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Clinical and Experimental

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THE SIGNIFICANCE OF CHOLESTEROL VARIATIONS IN HUMAN BLOOD SERUM

LESTER M. MORRISON, M.D., WILLIAM T. GONZALES, M.D., AND
LILLIAN HALL, M.D.
LOS ANGELES, CALIF.

IT HAS been pointed out that the literature dealing with serum concentrations of lipids and cholesterol is filled with conflict.[1, 2] These inconsistencies appear to be accounted for by differences in technique and the unreliability of certain methods now widely used. This subject has been reviewed adequately elsewhere.[3, 4] Accordingly, a modification of the Sperry-Schoenheimer method[4] has been employed for cholesterol, as developed by Chaney and Lovell.[5] This modification has been checked against the original Sperry-Schoenheimer method and also against other procedures in wide use. The results again emphasize the need for adhering to the fundamental principles of the Sperry-Schoenheimer procedure and demonstrate that wide variations in results are likely to occur in other methods which do not adequately control the Liebermann-Burchard color reaction. The modification of Chaney and Lovell appreciably curtails the time required for the procedure and reduces the complexities of the original Sperry-Schoenheimer method while maintaining its accuracy.

The need for standardization and perfection of such technique has been even more emphasized by the recent renewal of interest in and investigations into the role of fat metabolism in the production of arteriosclerosis and in particular coronary thrombosis. Recent studies[2] suggested that serum cholesterol levels are subject to variations under certain conditions in the same subject at different time periods. We therefore undertook an investigation into these circumstances of variation and present the following results.

The additional value of such an inquiry would be in supplying further criteria on which to evaluate the role of certain lipotropic agents, such as choline, inositol, etc. These have been recently employed both experimentally and clinically as "decholesterizing" agents in arteriosclerosis.[6-9] Changes in serum cholesterol values were reported[8] following the use of these agents.

CLINICAL MATERIAL

Two or more fasting serum cholesterol tests were made at intervals in 161 subjects who were divided into four groups. Group A consisted of thirty-two normal individuals who were free of any clinical symptoms or demonstrable evidence of any illness. These subjects served as the normal controls. Group B was selected as a control group comprising thirty-one patients who had varied illnesses such as chronic peptic ulcer, chronic

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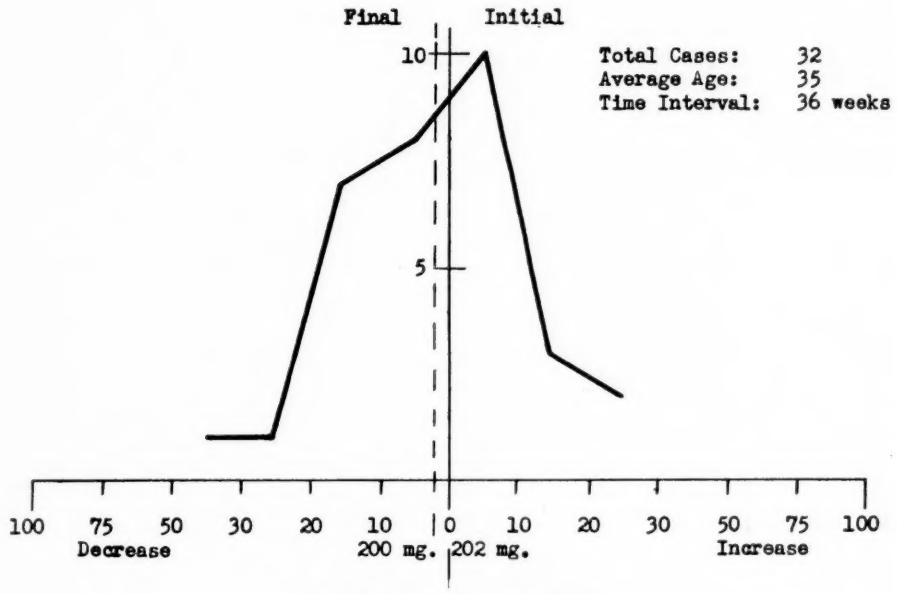
nephritis, chronic nonspecific ulcerative colitis, cirrhosis of the liver, chronic, rheumatoid arthritis, and essential hypertension. No patient in this group had known evidence of coronary occlusion or angina pectoris. Group C consisted of fifty patients who had had recent coronary occlusions (within a six-month period of time) but who received no specific medication or lipotrophic agents, except in a few instances where maintenance doses of digitalis or Mercuhydrin had been prescribed. Group D comprised forty-eight patients who had been on 6 Gm. daily doses of choline bicarbonate for variable periods of time and who had suffered a recent coronary occlusion within six months of the institution of choline therapy. As in Group C, several patients were on maintenance doses of digitalis or Mercuhydrin.

RESULTS

Group A.—Thirty-two normal subjects were studied whose ages ranged from 13 years to 72 years, the average age being 35 years. Twenty were male subjects; twelve were female. The time interval between the first cholesterol determination and the second (which was the last in most instances) ranged from eight weeks to sixty-four weeks, the average time interval being thirty-six weeks. As shown in Fig. 1, the average initial serum cholesterol was 202 mg. and the average final serum cholesterol was 200 milligrams. The variations between these two ranged from 0 to 38 mg., with an average variation of 11 milligrams. The normal serum cholesterol values are from 140 to 220 milligrams. Thus Fig. 1 illustrates the constancy of cholesterol levels in normal controls, when two or more determinations separated by a considerable period of time (one to sixty-four weeks) are made on the same individual. It will be noted that the increase or decrease in level is slight, less than 20 mg. per cent (or about 10 per cent of the total) in nearly all instances.

Group B.—This group consisted of thirty-one patients with various diseases as noted, excluding known illness due to coronary artery disease. The ages ranged from 24 years to 62 years, the average being 46 years. Twenty patients were men; eleven were women. As shown in Fig. 2, miscellaneous patient controls, the average initial cholesterol was 279 mg. and the average final cholesterol was 280 milligrams. The intervals of observation ranged from one week to twenty-six weeks, the average being nine weeks. In this group were twelve patients whose serum cholesterol varied less than 10 per cent between the first and last determinations, the average amount of cholesterol variation being 23 milligrams. There also were ten patients whose serum cholesterol values showed substantial decreases, on an average of 83 mg. between the first and last determinations. There were an additional nine patients whose serum cholesterol increased on an average of 73 mg. between the first and last determinations. In Fig. 2, describing this miscellaneous series of conditions, some involving elevated cholesterol values, but in which coronary artery disease was absent, the tendency for greater fluctuations to occur is evident from the graph, and increases or decreases up to 100 mg. or more occur as frequently as lesser deviations.

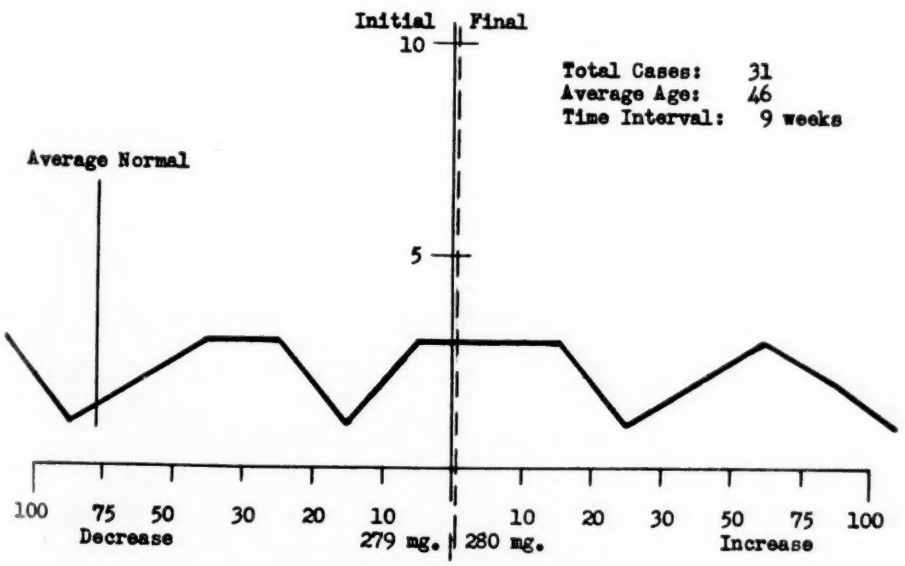
Group C.—Group C consisted of fifty patients who had had recent coronary artery occlusions (coronary occlusion controls), Fig. 3, within six months prior to the study. Their ages ranged from 38 years to 80 years, the average being 62 years. Forty patients were men; ten patients were women. The interval



Cholesterol Level Changes

NORMAL CONTROLS

Fig. 1.



Cholesterol Level Changes

MISCELLANEOUS PATIENT CONTROLS

Fig. 2.

range between initial and final cholesterol determinations was from one week to sixty weeks, the average being fourteen weeks. In this group there were three further subgroups. Ten patients had less than 10 per cent variations in serum cholesterol from first to last determinations, the average variation being 26 milligrams. Nineteen patients showed substantial interval cholesterol decreases with an average decrease of 69 milligrams. Twenty-one revealed interval

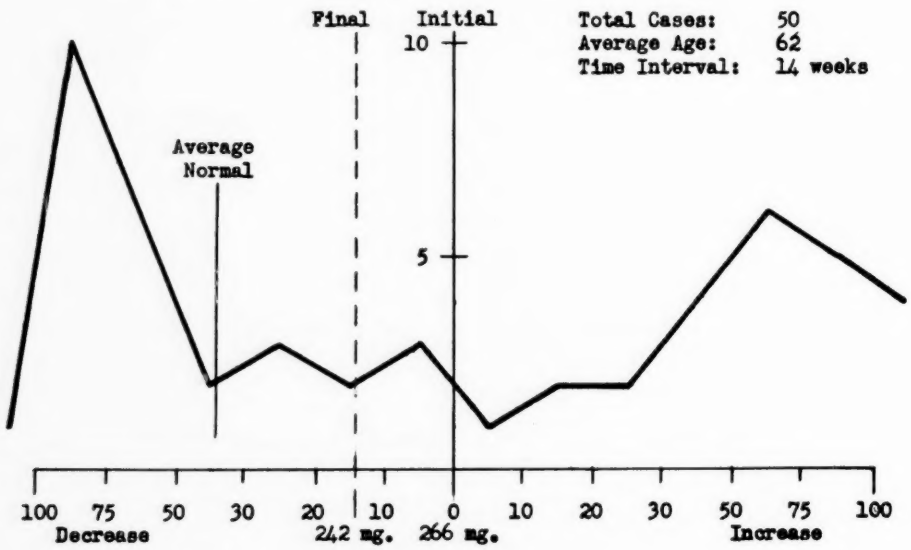


Fig. 3.

cholesterol increases with an average of 83 mg. increase. Fig. 3 (coronary occlusion controls) reveals that the average initial serum cholesterol was 266 mg. and the average final serum cholesterol was 242 milligrams. In acute coronary occlusion, which condition is frequently associated with elevated blood cholesterol, as has been shown previously,[2] there is a more marked tendency to fluctuate. Here it is noted that the majority of cases show a marked difference in level from an initial determination to a succeeding one and that this difference may as frequently be an increase as a decrease.

Group D.—This group consisted of forty-eight patients who had had recent coronary artery occlusion within six months prior to testing and treating, most of whom had received 6 Gm. of choline bicarbonate daily. This was given in three divided daily doses, each containing 2 Gm. (coronary occlusion—choline therapy, Fig. 4). The ages ranged from 29 years to 80 years, with an average age of 59 years. Forty-two patients were men and six patients were women. The interval range between the first and last cholesterol while on choline therapy was from one week to sixty-seven weeks, the average being twelve weeks. This

group had three further subgroups. One consisted of thirteen patients who showed less than 10 per cent interval variation in serum cholesterol values. The average variation was 5 milligrams. Eighteen patients revealed an average interval decrease of 72 mg. while on choline therapy. Seventeen patients showed an average interval increase of 98 mg. on choline therapy. Fig. 4 further shows how these fluctuations render more difficult the interpretation of the effect of

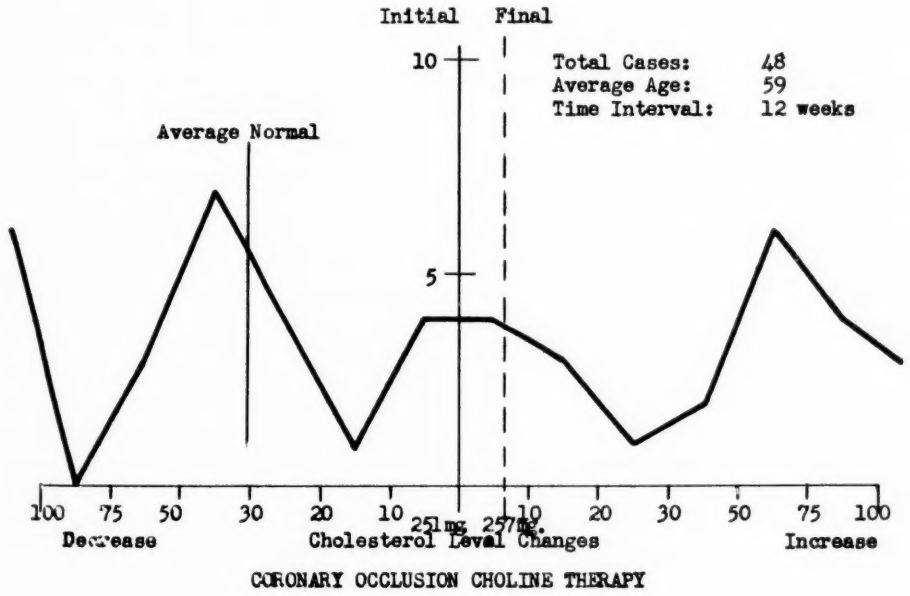


Fig. 4.

choline feeding on cholesterol levels. However, by graphing the results on this series (Fig. 4) in the same manner, it is apparent that no significant change can be attributed to the choline which is not already accounted for by the fluctuations inherent in the disease.

DISCUSSION

Confirmation is again made of Sperry's observation that in normal health the blood serum cholesterol is maintained at a constitutional level characteristic for the individual and from which significant alterations are not prone to take place. It is of interest to note here that in two of the normal control subjects, wider variations in serum cholesterol were found than in the other subjects. Upon careful interrogation, it was elicited that one subject, aged 41, had had a severe attack of infectious hepatitis eighteen years previously. Liver function tests in this individual had shown moderate impairment up to five years following recovery but had not been repeated, since the subject felt himself to be in good health. After the serum cholesterol determinations had been made and were found to be more variable than in the other normal subjects (a variation of 29 mg.), liver function tests were repeated and showed again low-grade impairment of liver function tests.

The other normal control subject, age 42, who revealed a 38 mg. variation, was closely questioned and was found to have a brother who had died recently of an acute coronary occlusion with hypercholesterolemia, one sister who had had a coronary artery occlusion, and parents who had died of coronary occlusions. A familial susceptibility or hereditary cholesterol metabolism disorder associated with coronary artery disease may be a possibility in this case.[13] Our control series of fifty cases of coronary thrombosis (Group C) confirms the findings of Steiner and Domanski[11] who also found that the serum cholesterol level in patients with coronary arteriosclerosis is variable and subject to wide fluctuations. Our finding of wide serum cholesterol fluctuations in miscellaneous diseases is in agreement with the report just published by Astrup.[12] The Group D patients with coronary artery occlusions who were under choline therapy revealed the same type of fluctuations of serum cholesterol observed in the control group of noncholine-treated coronary artery occlusion cases. That is to say, there were three similar subgroups, one showing 10 per cent fluctuation in serum cholesterol values on choline treatment, another showing significant increases in serum cholesterol values on choline treatment, and the other showing significant decrease under therapy.

It has been demonstrated that the liver exerts the chief control over cholesterol metabolism, as well as fat metabolism.[1] It also has been established that the liver plays the main role in the synthesis, distribution, and regulation of cholesterol in the blood stream. It is therefore possible that abnormal variations or instability in the serum cholesterol levels encountered in Groups B and C, comprising coronary thrombosis and certain other diseases, indicate an impairment in the function of the liver regulating cholesterol metabolism in these diseases.

SUMMARY AND CONCLUSIONS

1. In a series of thirty-two normal subjects, the constancy of blood serum cholesterol levels was reaffirmed over prolonged test periods using a modification of the Sperry-Schoenheimer serum cholesterol procedure.

2. Wide variations in serum cholesterol values were found in a group of thirty-one patients with miscellaneous diseases.

3. Marked fluctuations in serum cholesterol values were observed in a series of fifty patients who had recently experienced a coronary artery thrombosis.

4. Analogous wide fluctuations in serum cholesterol values were found in a series of forty-eight patients who had recently experienced a coronary artery thrombosis and ingested 6 Gm. of choline daily. These fluctuations rendered it impracticable to determine whether choline effected a reduction or increase in serum cholesterol levels.

5. It is suggested that variations or instability in serum cholesterol exceeding 15 per cent when determined by the Sperry-Schoenheimer procedure or a proved modification thereof in an individual presumed to be normal may possibly indicate a systemic disorder or latent illness.

6. It is suggested that if the Sperry-Schoenheimer procedure or a proved modification thereof shows abnormal variations in serum cholesterol, these may be due to a disturbance in the function of the liver regulating cholesterol metabolism.

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IRON METABOLISM

Erythrocyte Iron Turnover

C. A. Finch, M.D.,* J. A. Wolff, M.D.,† and C. E. Rath, M.D.,‡ Boston, Mass., and R. G. Fluharty, Ph.D.,§ Cambridge, Mass.

THE hemoglobin cycle in man is particularly accessible to studies employing radioactive isotopes of iron. The incorporation of radioiron into circulating hemoglobin or the disappearance of tagged cells from circulation may be measured. Such studies have necessarily been of short duration. In the turnover of the red cell mass, iron liberated from hemoglobin is passed on to other tissue and serum proteins (Fig. 1) and rapidly rerouted to the bone marrow. Once the radioiron reaches a state of equilibrium in the circulating red cell mass, the level of radioactivity may remain relatively constant for months or years. In the present study it has been possible to isolate the erythrocyte portion of the hemoglobin cycle, so that the survival of a population of red cells of approximately the same age might be determined.

The assumption has been made on the basis of in vitro studies[1, 2] that circulating red cells neither take up nor give off iron. The reticulocyte, however, has recently been shown to take up iron and synthesize heme.[3] These studies have allowed further observations in vivo and over a long period of time regarding the question of iron exchange between red cell and plasma.

Certain observations have previously been made regarding the breakdown and reutilization of hemoglobin iron. In studies on blood preservation, donors were prepared by injecting radioiron and obtaining blood after the iron had been incorporated in the red cell mass. The disappearance rate of these tagged erythrocytes from the blood stream of the recipient was measured over a period of twenty-four to forty-eight hours.[4-7] Thereafter, the radioiron was rapidly incorporated into new red cells so that in the ensuing days the level of radioactivity approximated that originally present. If red cell destruction occurred more gradually, no drop in the circulating radioactivity level was observed. This efficient reutilization of broken down hemoglobin iron has been commented on by several investigators as indicating that iron so liberated from hemoglobin falls into a small labile reserve which is rapidly recirculated through the hemoglobin cycle.[8, 9, 10] It has been shown that this utilization of radioiron for hemoglobin production may be largely prevented under two circumstances:[11]

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first, when the bone marrow is producing little or no blood, and second, when the iron stores are greatly enlarged. Advantage was taken of this in the choice of recipients for our studies. Subjects were selected who for one of these reasons should be unable to reutilize the radioiron liberated from tagged erythrocytes. It would then be possible to follow the intraerythrocytic radioiron through the period of time that the red cell remained in the circulation, provided no free exchange of iron occurred.

PROTEIN-IRON COMPLEXES OF THE HEMOGLOBIN CYCLE

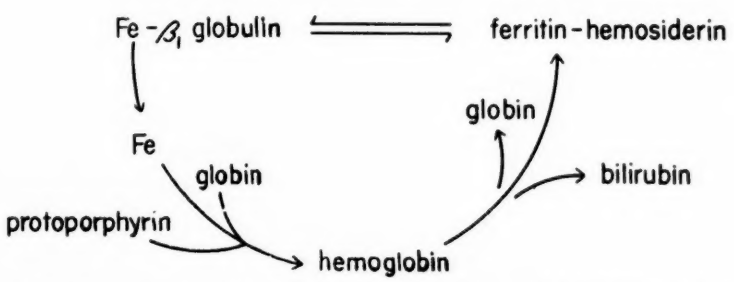

Fig. 1.—In the serum, iron is bound to a beta$_1$ globulin which transports iron from one tissue to another within the body. In the marrow the iron is stripped from the beta$_1$ globulin by the developing red cell and synthesized along with pyrol pigment and globin into hemoglobin. As hemoglobin, the red cell iron would appear to remain within the erythrocytes until the stroma is broken down. At this time, the pigment is degraded into its component parts and the iron may reappear in the serum bound to the transport globulin or may remain in the tissues incorporated in one of the iron storage proteins, ferritin or hemosiderin.

MATERIALS AND METHODS

Single isotopes of Fe^{55} and Fe^{59} obtained by cyclotron bombardment at the Massachusetts Institute of Technology were employed in both human and animal studies. Radioactive iron containing both isotopes Fe^{55} and Fe^{59} with a specific activity of 20 and 80 microcuries per milligram of iron, obtained from Oak Ridge, was used in some animal experiments. Radioactive donors were prepared as previously described.[2] Tagged blood for transfusion was drawn into acid citrate dextrose preservative and given immediately to the recipient except in one instance in which the effect of storage was under study. The circulating level of radioactivity approximately twenty minutes after blood was given to the recipient was determined and taken to represent 100 per cent of the transfused cells. In two instances, however, where subsequent levels were significantly higher, the latter values were assumed to represent 100 per cent. The blood volume as determined by T1824 dye was compared with the cell volume as determined by dilution of the transfused tagged cells. Subsequent blood samples were taken into calibrated centrifuge tubes over a period of 150 days. Hematocrits were determined on an aliquot by the Wintrobe method. The subsequent wet ashing of samples, addition of carrier iron, precipitation, electroplating, and counting have been described previously.[12]

EXPERIMENTAL DATA

I. Animals.—Dog S-FD90 was given 0.2 mg. Fe^{55} (1×10^6 counts per minute) intravenously and the subsequent incorporation of the iron into the circulating red cell mass was determined (Fig. 2). At fifteen days 75 per cent

of the injected iron was found in the peripheral blood.* On the twenty-seventh day and until the 108th day injections of nonradioactive iron in the form of ferrous-ascorbate gelatin† totalling 4,170 mg. were given intravenously. Throughout the period of observation the dog remained in good condition.

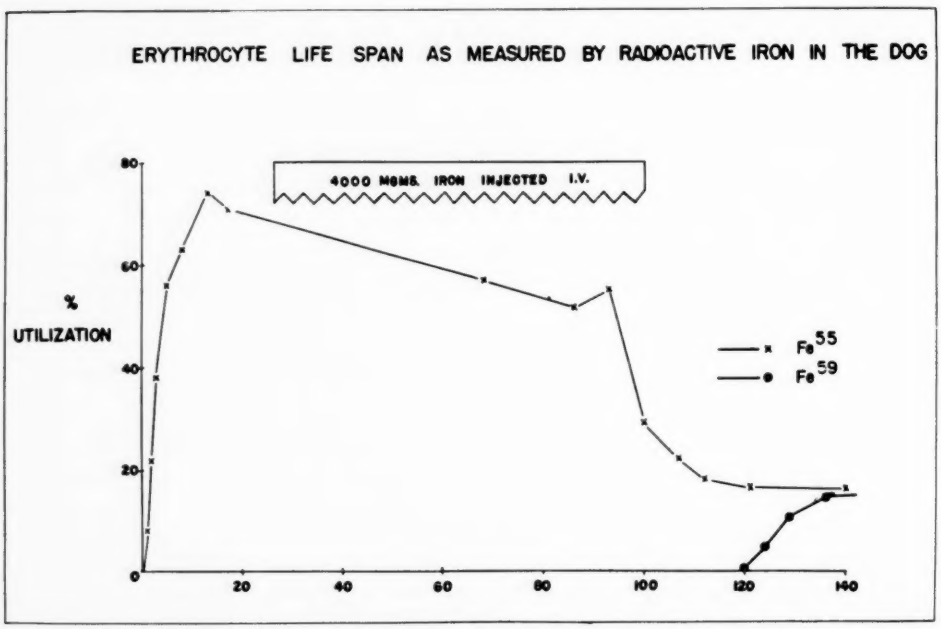

Fig. 2.—The initial utilization of Fe^{55} in a normal dog is shown. Circulating radio levels were followed during a period of 100 days while nonradioactive iron was injected intravenously. A subsequent utilization curve with Fe^{59} is shown. The ordinate is expressed in days.

There was a gradual drop in hemoglobin from 18 to 15 grams but no change in cell indices, reticulocytes, or in the leucocyte picture. In Fig. 2 is shown the level of circulating radioactivity. At 120 days 0.5 mg. of Fe^{59} (1×10^6 counts per minute) was injected intravenously. Sixteen per cent of this iron appeared in the red blood cell mass at the end of two and a half weeks.

In two further experiments dogs were placed on corn grit and iron diets which had previously been shown to produce large iron stores in animals.[13] These dogs received red cells prepared by injecting radioiron in a single donor dog one week previously. A similar rapid decline in circulating radioactive levels was observed in these dogs, in the neighborhood of 100 days. The red cell life was calculated from the time 50 per cent of the tagged cells had appeared in the blood of the donor dog to the time 50 per cent of the circulating radioactivity had been lost from the blood of the recipient dogs. In Dog J-FD219 the erythrocyte life span was 106 days and the average survival in Dog C-FD218 was 109 days. These dogs showed no significant hematologic

*The dog's blood volume was determined by T1824 dye and the per cent circulating radioactivity calculated as previously described[11] according to the formula: per cent activity = counts per cubic centimeter red cells × cubic centimeter circulating red cells divided by the counts injected.

†This iron preparation was obtained through the courtesy of the Knox Gelatine Protein Products, Inc., Camden, N. J.

change through the period of experimentation. The pathologic examination of both dogs revealed extensive deposits of hemosiderin and greatly increased tissue iron by analysis.

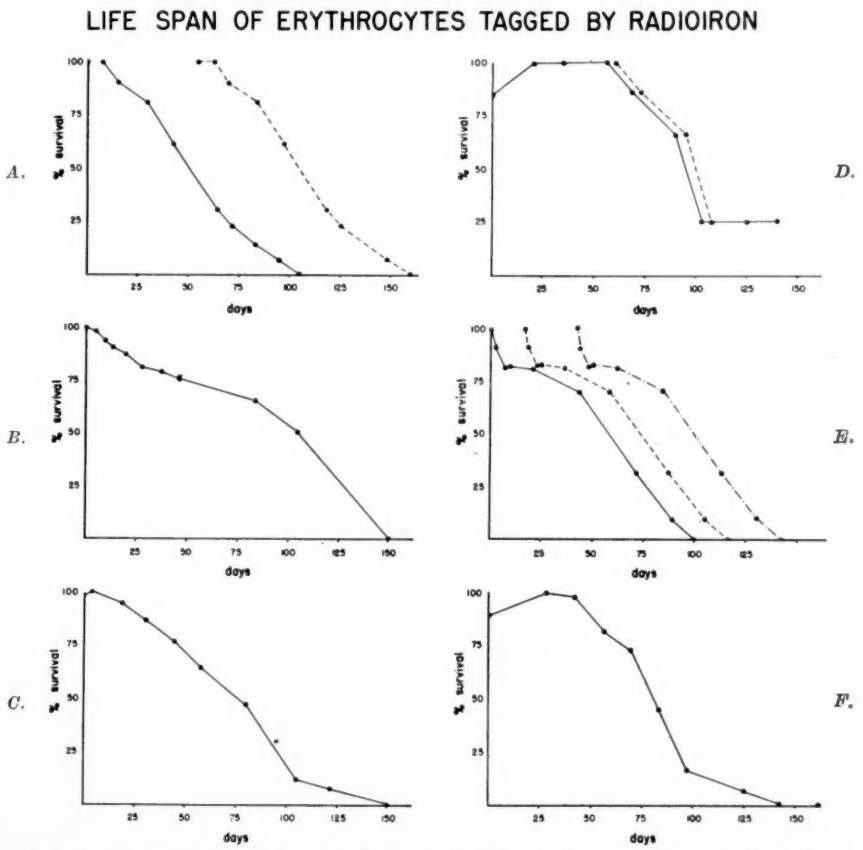

Fig. 3.—The solid line represents the actual radioactivity levels in the circulating blood of the recipients. The dotted line in *A*, *D*, and *E* allows for the added period of red cell life in the donor. In *E*, the third line represents the additional period of blood storage.

A, Sixty-day-old normal cells to patient with aplastic anemia.
B, Pernicious anemia reticulocytes to patient with aplastic anemia.
C, Iron-deficient reticulocytes to patient with aplastic anemia.
D, Iron-deficient erythrocytes to a patient with hemochromatosis.
E, Twenty-one-day-old blood stored for twenty-four days transfused to a patient with Cooley's anemia.
F, Iron-deficient reticulocytes transfused to a patient with Cooley's anemia.

II. Human Subjects.—Six populations of radioactively tagged erythrocytes were followed in recipients chosen because of their large iron stores and/or impaired bone marrow function. There follows in each instance a characterization of the blood given, the age of the red cells given, the hematologic status of the recipient, and the radioactive survival curves as determined by measuring levels of radioactivity in the recipient blood. The data are plotted in Fig. 3.

A: Five hundred cubic centimeters of blood were drawn from a young man who had been given radioiron (Fe^{55}) sixty-five days previously. The donor's red cells were normal in appearance and measurements showed a mean

corpuscular volume (MCV) of 85, a mean corpuscular hemoglobin (MCH) of 32, and a mean corpuscular hemoglobin concentration (MCHC) of 35. The recipient W-FD129 was a girl of 6 years with aplastic anemia who had been transfused every six to eight weeks since the age of 2 months. Preceding the transfusion her blood showed a hemoglobin of 5 grams, red count 1.8 million, and hematocrit 16 (MCV 108, MCHC 28, MCH 31) and no reticulocytes in 1,000 red blood cells. The subsequent decline in circulating radioactivity levels is shown in Fig. 3, *A*. The projected life span of the red cells, allowing for the time they had circulated in the donor, is shown by the dotted line.

B: The same recipient, W-FD129, was transfused one month later with 250 c.c. of blood tagged with Fe^{59}. The donor in this case, G-FD131, had classical pernicious anemia and had received liver six days previously and radioiron four days before the blood was drawn. The transfused blood had a MCV of 130, MCH 37, and MCHC 35. There were 31.9 reticulocytes. In the blood fractionated by albumin flotation[14] the distribution of radioactivity corresponds to the distribution of reticulocytes (Table I). These data would indicate that the isotope is found almost exclusively in the reticulocyte fraction and that, in effect, tagged reticulocytes are being transfused. Subsequent survival of this blood is shown in Fig. 3, *B*.

C: Recipient W-FD129 was used for a third study after all previously administered radioactivity had disappeared from her circulating blood. Donor SL-FD262 had typical untreated polycythemia vera. The donor's blood showed a hemoglobin of 20.1 grams with slight microcytosis and hypochromia (MCV 75, MCH 23, MCHC 31). Five hundred cubic centimeters of blood were drawn on the third day after injection of radioiron. Evidence that these tagged cells were comprised chiefly of reticulocytes is shown in Table I. The determinations of circulating radioiron levels in the recipient are shown in Fig. 3, *C*.

D: Five hundred cubic centimeters of blood were drawn from the same donor, SL-FD262, on the tenth day following injection of radioiron. This blood was transfused to recipient R-FD108 who had hemochromatosis confirmed by

TABLE I

IRON DEFICIENT BLOOD	COUNTS/UNIT CELLS	RETICS (%)
Top fraction	2,900	10.8
Middle fraction	920	2.9
Bottom fraction	500	1
PERNICIOUS ANEMIA BLOOD		
Top fraction	5,660	39.4
Bottom fraction	187	0.1

liver biopsy. The recipient had a saturated iron binding protein, a serum iron of 250 μg per 100 c.c., and a slightly macrocytic anemia (hematocrit 40, MCV 102). Radioactivity was expressed as counts per cubic centimeter of red cells since it was felt that the red cell mass should be relatively constant through the period of observation. This is in contrast to the other cell viability experiments in which activity is related to cubic centimeters of whole blood. There is no evident explanation for the initial lower level of radioactivity unless it is

related to the temporary red cell mass increase due to the transfusion. It is to be noticed (Fig. 3, *D*) that there is a reutilization in this patient of 25 per cent of the erythrocyte iron similar to that seen in previous animal experiments.

E: A third phlebotomy was performed on the donor on the twenty-first day after iron injection. This blood was preserved in acid citrate dextrose for twenty-five days at 4° to 6° C. and then transfused. The recipient, JS-FD265, was a 4-year-old child who had Cooley's anemia and had received approximately twenty transfusions before the present study. On the day of transfusion his hemoglobin was 10 grams. The survival time of these red cells and the projected survival allowing for their time in the donor and preservative are shown in Fig. 3, *E*.

F: Donor LK-FD244 was a patient with polycythemia vera who had been controlled by phlebotomy for two years. He showed a high-grade iron deficiency at the time of phlebotomy (MCV 70, MCH 22, MCHC 30). Six days after injection of Fe^{55}, 500 c.c. of blood were removed and transfused immediately to recipient L-FD263. The subsequent levels of circulating radio-iron per cubic centimeter of whole blood are shown in Fig. 3, *F*. The recipient in this transfusion was a 4-year-old girl who had been anemic since birth with typical findings of Cooley's anemia. She had had more than eight transfusions before the present study and a hematocrit of 18.2 prior to transfusion.

In another series of observations, normal male subjects were given Fe^{55} intravenously and beginning twenty-one days later iron was taken for four to six months by mouth immediately after meals. Subjects F and R consumed 200 Gm. of ferrous sulfate and Subject M 400 grams. It would appear that enough iron was absorbed in this fashion to interfere somewhat with reutilization and to allow one to approximate the life span of the erythrocyte in each case (Fig. 4). Mean cell age was calculated as 125 days (Subject F), 125 days (Subject R), and 114 days (Subject M).

DISCUSSION

The sensitivity of the counters used reach 3 per cent efficiency in the case of Fe^{55} and 20 per cent in the case of Fe^{59}. The counting levels obtained from blood samples were sufficient to allow accurate measurement of samples containing less than 5 per cent of the initial circulating radioactivity except for experiment B (Fig. 3, *B*). Here, accurate counting, that is counts more than twice background, was attained only in values showing over 30 per cent erythrocyte survival.

In the dogs and in the patient with hemochromatosis (Figs. 2 and 3, *D*) there was appreciable reutilization of the erythrocyte iron, in the neighborhood of 25 per cent. Since these new base lines are stable over a period of several weeks, it seems justifiable to assume 100 per cent destruction of transfused erythrocytes when this level has been attained. In the remaining transfusions there was no significant reutilization. Normal subjects fed iron, however, showed so great a reutilization (approximately 75 per cent) that interpretation of the data is difficult.

In these studies a population of red cells of approximately one age has been followed throughout their survival period in the circulating blood. The entrance of these cells into the circulation in the donor is shown by the utilization of the injected iron for hemoglobin production. In Fig. 5 is a composite curve of such utilization of radioiron obtained previously in normal subjects. This

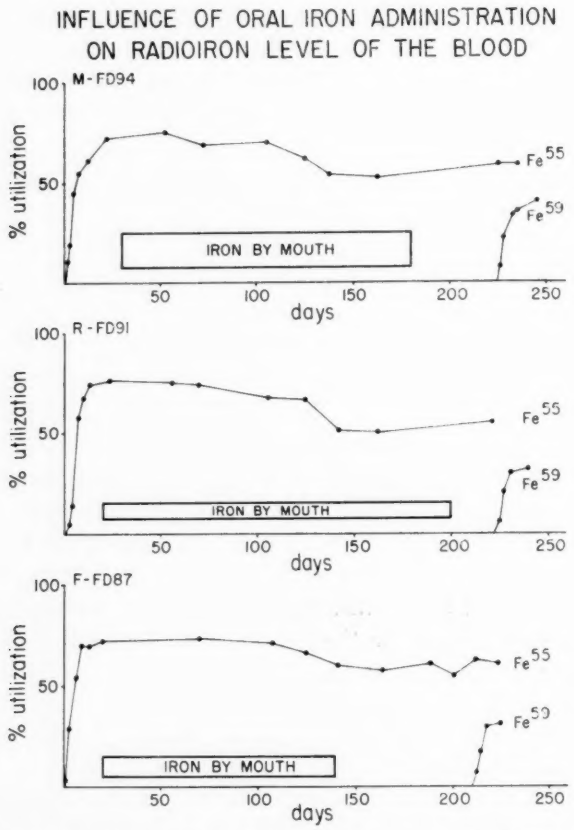

Fig. 4.—The initial utilization of radioiron in three normal subjects is shown and the subsequent circulating radioactive levels during the period of iron intake by mouth. The subsequent utilization curve with Fe^{59} is also shown. It will be observed that between 100 and 150 days there is a significant decline in circulating radioactivity levels in each subject.

indicates that normally the fourth day will represent the mean age of tagged cells and that some 75 per cent of the total number of tagged cells appear in circulation between the second and seventh days.[11] In iron-deficient and pernicious anemia patients responding to treatment, the utilization may be even more rapid. For purposes of calculation in Fig. 3, corrections are made by a dotted line for the mean age of the cells, according to when they would be expected to appear in the donors circulation.

It might be questioned whether the sample taken immediately after transfusion is representative of the initial red cell level or whether during the process of transfusion which took between one-half and two hours some red cells

might already be lost from the blood stream. It has been previously demonstrated that nonviable erythrocytes may disappear very rapidly from circulation, that is, within minutes of transfusion.[4, 15] For this reason it appeared necessary to have some check on the initial level. Highly quantitative measurements were not possible since the blood given was only roughly measured and

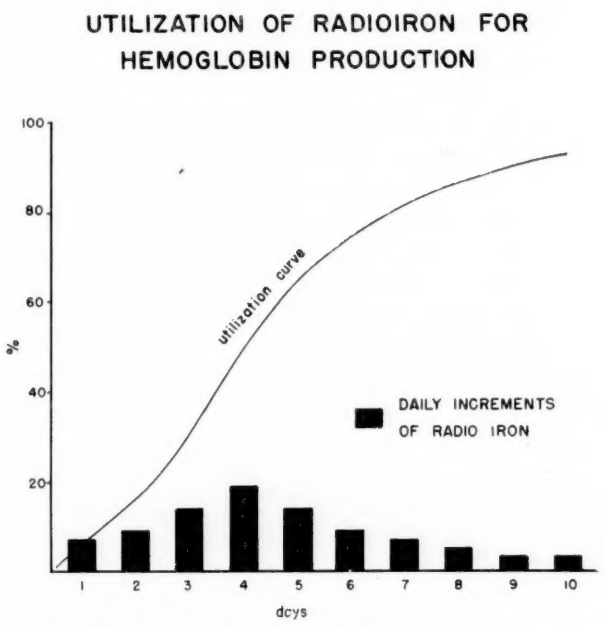

Fig. 5.—On the basis of data previously reported[11] a composite utilization curve is shown. This has been normalized from the 74 per cent which appears in the blood to 100 per cent so that the daily increment of radioiron incorporated in erythrocytes may be shown.

since the transfusion set was not always completely emptied of blood. In patient FW-FD129 three transfusions were given (Fig. 3, *A*, *B*, and *C*). The calculated blood volume on the basis of the radioactivity administered and the initial post-transfusion level were, respectively, in observations A, B, and C, 1,340 c.c., 1,400 c.c., and 1,280 cubic centimeters. Since A represents fresh normal cells which have been shown previously to be a reliable measure of the circulating mass,[16] the immediate survival of the other two populations of red cells may be assumed to be reasonably good. In D, the calculated radioactive cell volume was 2,160 c.c. while the Evans blue dye determination gave a cell mass of 1,975 cubic centimeters. In E, a blood volume employing T1824 immediately after transfusion gave a determination of 1,030 c.c. whereas the radioactive blood volume was 950 cubic centimeters. In patient L-FD263 (F) an Evans blue blood volume performed six months after the initial study was 4,700 c.c. as compared with the post-transfusion radioactive measurement of 4,200 c.c. in the same patient. In no instance, therefore, was there an indication of an abnormally large blood volume by radioactive measurement using the twenty minute samples. This would appear to exclude any appreciable cell destruction during the time of transfusion since such an event would result in a greatly enlarged cell volume determination by the radioactive method.[15]

Previous work has shown that very little radioiron injected intravenously appears in the circulating red cell mass when bone marrow synthesis is severely impaired, suggesting that only as a result of hemoglobin synthesis by the developing red cell can radioiron gain access to the erythrocyte.[10, 11] In the present studies, reutilization of iron is largely or completely blocked. Should there be an exchange of hemoglobin iron with plasma iron, the level of circulating activity would rapidly fall. The radioactivity in the recipients' circulation, however, followed a survival curve similar to that obtained when the pyrrol pigment[17] or globin of the hemoglobin molecule is isotopically tagged. It would then appear to be a safe assumption that the mature red cell throughout its life span has no appreciable exchange of iron with its surrounding environment.

In three dogs the average turnover of iron from the time of appearance in circulation to disappearance is from 95 to 109 days. In patients transfused with various popuations of erythrocytes, 50 per cent of the erythrocytes had disappeared from circulation between 77 and 110 days after their appearance in circulation. The one normal cell population (Fig. 3, *A*) showed a mean cell survival of 110 days. This is consistent with other data of the life of the human red cell[18] and would indicate that normally there is approximately a 1 per cent daily turnover of iron through the red cell mass as a result of erythrogenesis and erythrocyte breakdown.

In contrast to differential agglutination studies in which the red cell population measured is a mixed one, in these studies red cells of a single age could be followed throughout their life period. By drawing blood shortly after injection of radioiron it was possible to obtain young cells as shown in Fig. 3, *B* and *C*. That these young cells represent reticulocytes is indicated by Table I and by other studies,[19] demonstrating that in fractionated blood the reticulocyte level and radioactive level show a high degree of correlation. Other studies[3, 20] have demonstrated that reticulocytes are capable of synthesizing heme and maturing, and that their disappearance from circulation is associated with an increase in mature red cells. The present observations would indicate that they not only mature but have a reasonably normal life span.

It is of interest that in pernicious anemia the reticulocytes after liver therapy have a normal life expectancy, since other observations on mixed population of untreated pernicious anemia macrocytes have indicated a considerably shorter life span. Iron deficiency reticulocytes as shown in Fig. 3, *C* and *F* survive a somewhat shorter than normal period, whereas the same cells in a subject with a relatively normal blood picture show a relatively normal survival. This suggests an influence of anemia per se on the life span although the varied nature of the recipients is such that no conclusions can be drawn. The different survival curves obtained with red cells from the same donor in different recipients (Fig. 3, *C* and *D*) emphasize the importance of such factors. It is of interest that storage of blood before transfusion seemed to result in an immediate loss of approximately 20 per cent of the transfused red cells while the remaining erythrocyte population survived what would appear to be a relatively normal life span.

In subjects fed iron the erythrocytes remained in their normal environment and were subjected to no manipulation. Although the great reutilization obscures the erythrocyte breakdown, the mean cell age estimated at 122 days is quite similar to the figure of 127 days obtained by Shemin and Rittenberg[17] using N^{15} in the same type of study.

CONCLUSIONS

The reutilization of radioactive iron from broken down red cells is effectively blocked by the presence of enlarged iron stores and/or bone marrow dysfunction in animals and in human subjects. By using recipients with these characteristics, the erythrocyte unit of iron metabolism may be isolated.

Under these experimental conditions it was possible to measure the life span of a red cell population of a single age. Observations were made of tagged macrocytes, microcytes, and normal red cells in normal and anemic recipients. The reticulocytes from a patient with pernicious anemia responding to therapy and from a patient with iron deficiency have been shown to have a relatively normal life span.

The erythrocyte iron turnover measured directly is approximately 1 per cent a day in dog and in man.

There is no discernible exchange of iron between the red cell and its surroundings throughout the life span of the erythrocyte.

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TYROSINE METABOLISM IN HUMAN SCURVY

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INTRODUCTION

IT HAS been shown that vitamin C is intimately connected with the metabolism of tyrosine and phenylalanine. Sealock and Silberstein[1] reported that when tyrosine was fed to scorbutic guinea pigs, homogentisic acid was excreted, a phenomenon that could be prevented by the administration of *l*-ascorbic acid. They also demonstrated[2] that tyrosine, fed to guinea pigs on a diet deficient in vitamin C, resulted in the excretion of homogentisic, *p*-hydroxyphenylpyruvic, and *p*-hydroxyphenyl lactic acids, and that the administration of *l*-ascorbic acid prevented the abnormal excretion of these metabolites. Painter and Silva[3] fed large doses of tyrosine to scorbutic guinea pigs but found no excretion of homogentisic acid in the urine, but did observe excessive excretion of the intermediary metabolites of tyrosine. In addition, Levine and coworkers[4, 5] noted that premature infants fed diets of vitamin C free cows' milk containing 5 Gm. or more of protein per kilogram of body weight excreted *p*-hydroxyphenyl lactic and *p*-hydroxyphenylpyruvic acids, the excretion of which was eradicated by the administration of ascorbic acid.

The purpose of this communication is to report the effects in normal and scorbutic human beings of feeding large amounts of tyrosine and the rapid effect of *l*-ascorbic acid in correcting the abnormal tyrosine metabolism found in the individuals with scurvy.

MATERIALS AND METHODS

The patients observed in this study were four scorbutic men aged 62, 64, 66, and 71 years, two normal men aged 22 and 29, and one normal woman aged 75.‡

During the course of the studies, the scorbutic patients were maintained on a vitamin C-free diet consisting of two quarts of boiled skimmed milk, 450 grams of boiled rice, and thirty-six soda crackers daily. Sugar, coffee, and vitamin C-free purified jellies were allowed ad libitum. The two normal male subjects were allowed their usual diets. The normal female subject was maintained on the same vitamin C-free diet as the scorbutic patients, but received large supplements of crystalline ascorbic acid. Twenty-four hour samples of urine were collected daily from these patients and after suitable control periods of at least six days, all except one scorbutic patient were given 20 Gm. of powdered *l*-tyrosine§ per day orally, divided into four doses of 5 Gm. each.

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‡This 75-year-old patient was thought to have scurvy because of purpura. The purpura was of senile type only, and she was then studied as a normal patient after receiving large supplements of ascorbic acid.

§Kindly supplied by Merck & Co., Inc., Rahway, N. J.

After a period of tyrosine administration, four of the scorbutic patients received large doses of ascorbic acid in addition to the tyrosine. When possible, a control period was included after the discontinuance of tyrosine.

The ascorbic acid content of the white blood cell-platelet layer was determined by the method of Butler and Cushman.[6] The addition of 100 mg. of human fibrinogen (Fraction I of human plasma [Cohn][7]) to 10 ml. of oxalated blood produced better separation of the white cell-platelet layer for assay. The whole blood ascorbic acid levels were determined by the method of Keuther.[8]

The total excretion of hydroxyphenyl compounds in the urine, expressed as tyrosine equivalents, was measured by the method of Medes.[9] The compounds measured by this method include tyrosine, *p*-hydroxyphenyl lactic, and *p*-hydroxyphenylpyruvic acids, and are designated "tyrosyl derivatives."

The reduction of phosphomolybdic acid by the urine as outlined by Medes[9] also was determined. This method, for the most part, measures the excretion of *p*-hydroxyphenylpyruvic acid, but is not specific and will react with homogentisic acid and other reducing substances. Since the reducing substances excreted by the scorbutic patients, while receiving tyrosine, were not chemically identified, their reducing power was calculated as equivalents of a standard solution of hydroquinone. The presence of homogentisic acid was tested for qualitatively by adding ferric chloride to urine that was made alkaline with sodium hydroxide. Calibration curves with hydroquinone standards for the phosphomolybdic reducing power and *l*-tyrosine for the "tyrosyl" derivatives were prepared using the Evelyn photoelectric cell colorimeter. The tyrosyl derivatives were read in the colorimeter exactly one minute after color formation to obtain maximum values. If they were read after allowing the color reaction to proceed for twenty minutes, as outlined by Levine and associates,[4] there was a marked drop in color intensity. The phosphomolybdic reaction was allowed to proceed for three hours before readings were made in the colorimeter.

RESULTS

Scorbutic Patients.—Case 1 (Fig. 1), a 62-year-old man, was admitted to the Boston City Hospital because of pain in the right thigh. He had classical signs of scurvy: a positive tourniquet test, purpura on the legs, ecchymoses of the right thigh, and hematomas around carious teeth. There was no detectable ascorbic acid present in the white blood cell-platelet layer of the blood. In a control period of six days on a vitamin C-free diet, this patient's total "tyrosyl" excretion averaged 195 mg. of tyrosine equivalents per day with a range of from 128 mg. to 248 mg. per day. In this same control period, the excretion of reducing substances as measured by the reduction of phosphomolybdic acid was equivalent to an average of 84 mg. of hydroquinone per day and ranged from 37 mg. to 146 mg. per day.

When the patient was given 20 Gm. of tyrosine daily, for six days, the excretion of "tyrosyl" compounds immediately increased, and on the sixth day of administration reached a level of 6,300 milligrams. The reducing substances of the urine remained unchanged from the control values for three days, but then steadily increased until on the sixth day of tyrosine administration the concentration was equivalent to 6,300 mg. of hydroquinone per day.

The patient was then given the daily addition of 1 Gm. of *l*-ascorbic acid orally as well as 20 Gm. of tyrosine per day for five days. The ascorbic acid was then reduced to 0.5 Gm. per day for the remainder of the study. With ascorbic acid therapy, the excretion of "tyrosyl" compounds immediately decreased and on the second day of therapy was 583 mg. and fluctuated between 453 and 1,073 mg. per day for the next seven days, when the study ended. The reducing substances of the urine showed no decrease on the first day of ascorbic acid therapy and, in fact, increased to 6,620 mg. of hydroquinone equivalents. However, on the second day of ascorbic acid therapy, the concentration of reducing substances dropped to slightly above control levels, averaged 152 mg. per day, and remained

at that value for four days. At this time the reducing power increased to a daily average of 252 mg. of hydroquinone equivalents and is interpreted as an increased excretion of ascorbic acid since this compound will reduce phosphomolybdic acid under the conditions used.

During the period of tyrosine administration, the capillary fragility increased, as measured by the tourniquet test, and there was some bleeding from the gingival hematomas. All signs of capillary fragility disappeared promptly after administration of ascorbic acid.

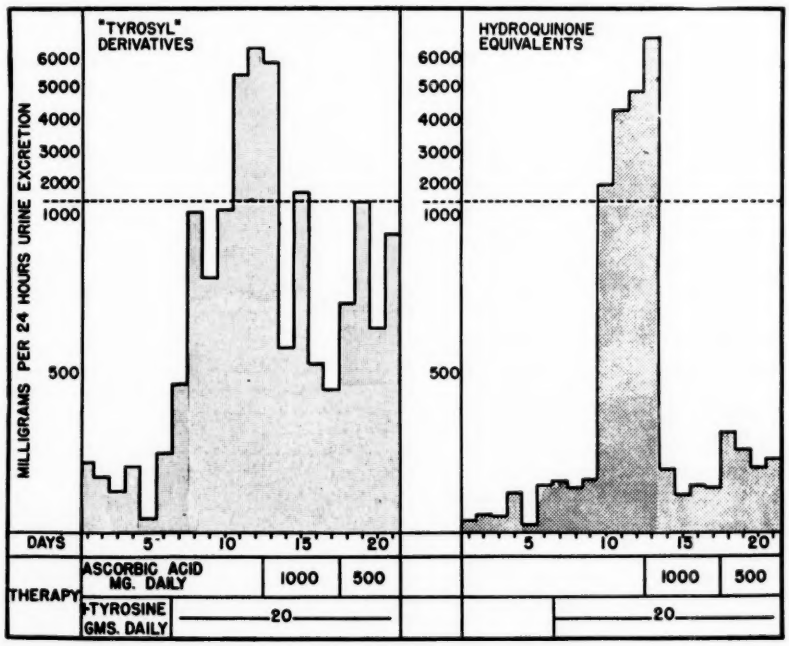

Fig. 1.—Case 1 (scurvy). Twenty-four hour excretion levels before and after treatment.

Case 2 (Figs. 2 and 3) was a 64-year-old man with the clinical picture of scurvy and with no detectable ascorbic acid in the white cell-platelet layer. He was maintained on the same vitamin C-free diet as provided for the first patient. In a nine-day control period he excreted a daily average of 109 mg. of "tyrosyl" compounds. The reducing substance of the urine in this same control period was equivalent to a daily average of 74 mg. of hydroquinone.

The patient was then given 20 Gm. of tyrosine daily orally, with a large immediate rise in excretion of "tyrosyl" derivatives. On the second day he excreted 7,700 mg., and on the fifth day, 16,120 milligrams. On the seventh day of tyrosine administration, he was given 2 Gm. of ascorbic acid orally per day. This dose was continued for five days and then decreased to 1 Gm. per day for the remainder of the observation period. Following the administration of ascorbic acid, the excretion of "tyrosyl" compounds rapidly fell and, although the patient received tyrosine for twenty-five days after institution of antiscorbutic therapy, his excretion of "tyrosyl" derivatives, after the initial drop became evident, averaged only 536 mg. per day with a daily range of from 200 to 1,400 milligrams. Tyrosine was stopped for a period of one week, during which time the average excretion dropped to 230 mg. per day. In order that the patient should act as his own control, he was given tyrosine for another eight-day period during which time the average excretion of "tyrosyl" derivatives was 312 mg. per day with the daily maximum being 400 mg. on the fourth day. In a final control period of ten days without tyrosine, he excreted an average of 140 mg. per day.

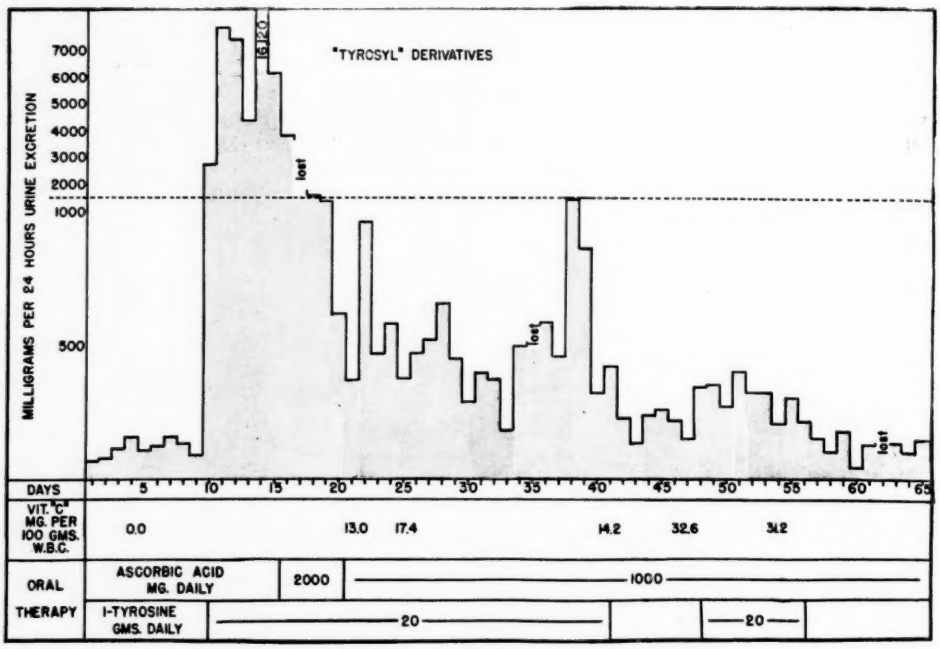

Fig. 2.—Case 2 (scurvy). Daily "tyrosyl" excretion.

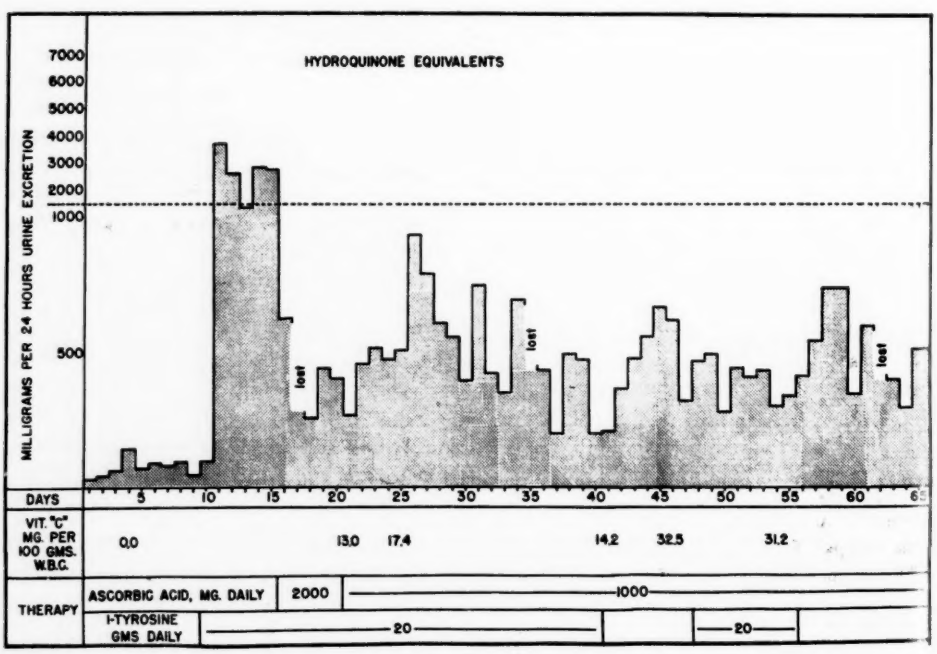

Fig. 3.—Case 2 (scurvy). Reducing substance in the urine measured as hydroquinone equivalent.

The reducing substances in this patient's urine, during the administration of tyrosine, increased rapidly to 3,480 mg. of hydroquinone equivalents on the second day and ranged above 2,500 mg. per day throughout this period except for one value of 1,340 mg. which may be in part an incomplete sample. There was a marked fall concomitant with the administration of 2 Gm. of ascorbic acid per day to an average level of 471 mg. of hydroquinone per day. Again it is believed that this higher level of excretion than in the original control period was caused by the excretion of ascorbic acid and was not due to the administration of tyrosine. This contention is borne out by the fact that when tyrosine administration was discontinued for seven days the daily average remained essentially the same (446 mg. of hydroquinone equivalents per day) and that on reinstitution of tyrosine administration the level tended to fall slightly, averaging 392 mg. of hydroquinone per day.

During the period of tyrosine administration, there was a marked increase in capillary fragility. For the first time, the tourniquet test produced numerous small hematomas over the forearm as well as petechial hemorrhages. The patient became weak and developed nausea and diarrhea; all symptoms disppeared when ascorbic acid was given.

Case 3 (Table I) was a 71-year-old man who entered the hospital with hematomas of both calves and marked capillary fragility. There was no detectable ascorbic acid in the white blood cell-platelet layer. Throughout the study the patient was on a vitamin C-free diet and received no additional tyrosine.

In a six-day control period, before the administration of ascorbic acid, the patient excreted an average of 201 mg. of hydroxyphenyl compounds per day, and the reducing substance of the urine was equivalent to 64 mg. of hydroquinone per twenty-four hours. After this control period, the patient was given 1 Gm. of ascorbic acid a day orally for five days and then 0.5 Gm. for the remainder of the study. With ascorbic acid therapy there was no significant change in excretion of "tyrosyl" derivatives which averaged 196 mg. per day. The reducing substances in the urine remained low (averaging 92 mg. hydroquinone equivalents per day) for four days and then rose to an average excretion of 192 mg. per day which undoubtedly reflected the increased amounts of ascorbic acid being excreted in the urine.

TABLE I. CASE 3 (SCURVY): EXCRETION LEVELS BEFORE AND AFTER ADMINISTRATION OF ASCORBIC ACID

TREATMENT	DAYS	AVERAGE TYROSYL DERIVATIVES (MG./24 HR.)	AVERAGE HYDROQUINONE EQUIVALENTS (MG./24 HR.)
0	6	201	64
Ascorbic acid:			
1.0 Gm. 5 days	10	196	192
0.5 Gm. 5 days			

Case 4 (Fig. 4). The patient entered the hospital complaining of weakness and inability to walk. He had large hematomas in the right popliteal fossa extending to the groin. There was no measurable vitamin C in the white blood cell-platelet layer of the blood. Throughout the observations the patient was maintained on the vitamin C-free diet. During a five-day control period the patient excreted a daily average of 219 mg. of "tyrosyl" derivatives. The average daily excretion of reducing substances in the urine was 20 mg. hydroquinone equivalents.

The patient was then given 20 Gm. of tyrosine daily. By the third day the "tyrosyl" excretion had increased to 11,300 mg. and the reducing substances to 3,025 milligrams.

During the period of tyrosine administration, the patient developed nausea, diarrhea, singultus, and marked lassitude. The diet intake was not maintained and urine collections could not be made. Tyrosine was discontinued and the patient received 1.5 Gm. of ascorbic acid parenterally and 2.5 Gm. orally daily. Despite the large doses of ascorbic acid and parenteral dextrose solution, the patient did not respond. He died five days after the onset of ascorbic acid therapy in an episode of pulmonary edema and shock. Post-mortem examination revealed only pulmonary congestion and no hemorrhagic phenomena.

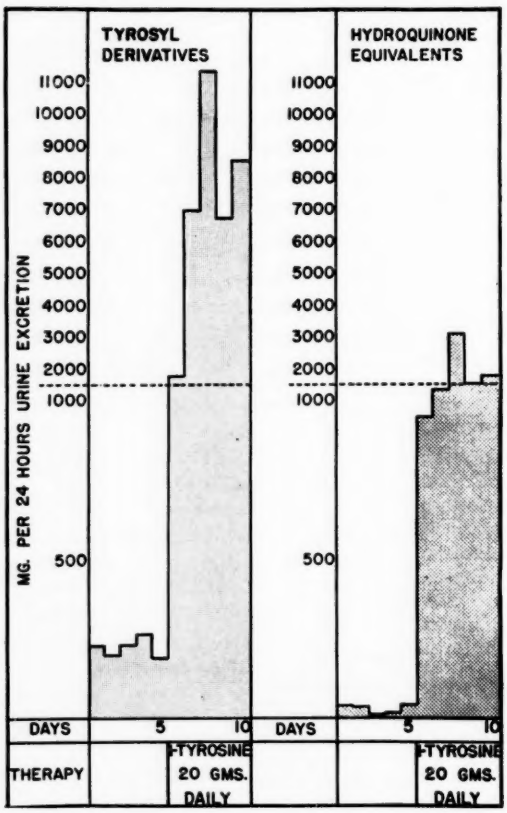

Fig. 4.—Case 4 (scurvy). Excretion levels during tyrosine administration.

TABLE II. RESPONSE OF THREE NORMAL SUBJECTS TO 20 GM. PER DAY OF L-TYROSINE ORALLY

PATIENT	L-TYROSINE (GM./DAY ORALLY)	AVERAGE TYROSYL DERIVATIVE (MG./24 HR.)	AVERAGE HYDROQUINONE EQUIVALENTS (MG./24 HR.)	DAYS IN PERIOD
Normal Subject 1	0	242	184	14
Period 1	20	755	167	11
(See Fig. 5)	0	287	135	6
Period 2*	0	328	533†	6
	20	734	588†	7
	0	290	171	5
	0	175	99	6
Normal Subject 2	20	614	112	9
	0	134	91	5
	0	218	683†	9
Normal Subject 3	20	582	887†	5
	0	230	636†	5

*500 mg. ascorbic acid given orally during control and administration of tyrosine.

†These values are markedly elevated because of ascorbic acid administration throughout this period.

Normal Patients.—Normal Subject 1 (Fig. 5) in a control period of fourteen days excreted an average of 242 mg. of "tyrosyl" compounds per day. The reducing substances in the urine in this same period were 184 mg. hydroquinone equivalents per day. He then received 20 Gm. of tyrosine daily for eleven days which resulted in a gradually increased excretion of "tyrosyl" compounds until the fifth day when it was 1,270 milligrams. Thereafter, for the next five days, a moderate fall in the excretion of "tyrosyl" derivatives occurred, followed by a second peak reaching 1,078 mg. on the last day of tyrosine administra-

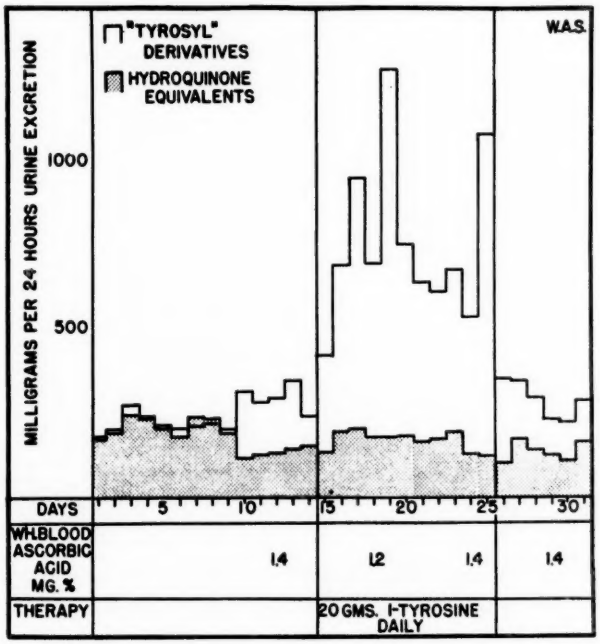

Fig. 5.—Normal Subject 1. Excretion levels of normal subject during tyrosine administration.

tion. The average excretion of "tyrosyl" derivatives during the entire period of tyrosine administration was 755 mg. per day; in a subsequent control period without tyrosine the excretion dropped to preadministration levels.

The reducing substances in this individual's urine did not change appreciably during any portion of the experiment. During the administration of tyrosine they were 167 mg. hydroquinone equivalents per day which was slightly lower than in the previous control period. Whole blood ascorbic acid levels were within normal limits and showed no significant change during the control periods or when the patient was receiving tyrosine.

In order to determine if added ascorbic acid would change the response to 20 Gm. of tyrosine per day, the same observations were repeated on this patient with the exception that he was given 500 mg. of ascorbic acid daily, starting three days before the administration of tyrosine and continuing through the entire period of tyrosine administration, Table II, period 2. The addition of ascorbic acid in this instance did not change the response to tyrosine as evidenced by the fact that on one day the patient excreted 1,134 mg. of "tyrosyl" derivatives and the average excretion during tyrosine administration was 734 mg. per day as compared with the value of 755 mg. per day when he was not receiving ascorbic acid. The reducing substances in the urine immediately increased when he took ascorbic acid, but were not augmented further on addition of tyrosine to his diet.

Normal Subject 2. The response to 20 Gm. of tyrosine daily for eight days in another normal male, aged 22, is shown in Table II. It is similar to that of the previously described normal person, although this patient excreted slightly less hydroxyphenyl compounds, averaging 614 mg. per day during the period of tyrosine administration. As in the first normal subject, after an initial rise, there was a decreased excretion for three to four days followed by a second rise.

Normal Subject 3, a 75-year-old woman, was given the same vitamin C-free diet as described for the scorbutic patients. In addition, she received 1 Gm. of ascorbic acid orally for the first eleven days of control observation and 2 Gm. daily throughout the remainder of the study. During a control period of nine days she averaged 218 mg. per day of "tyrosyl" derivatives. She was then given 20 Gm. of tyrosine daily for a five-day period and the "tyrosyl" excretion averaged 582 mg. per day. During another five-day control period the excretion averaged 230 mg. per day.

During a nine-day control period while receiving 2 Gm. of ascorbic acid daily, the patient's average excretion of reducing substances was 683 mg. hydroquinone equivalents. During the period of tyrosine administration it rose to a daily average of 887 milligrams. In the second five-day control period the daily average was 636 milligrams. Again it is believed that the high values in the control period are associated with the reducing power of ascorbic acid. This normal subject showed a rise in reducing power of the urine during tyrosine administration. Although no complete explanation is available, the large dosage of this amino acid may have materially aided in the excretion of ascorbic acid.

DISCUSSION

From the foregoing results, it would seem safe to assume that the relationship between ascorbic acid and the metabolism of tyrosine in the adult human being is similar to that which had previously been demonstrated in the guinea pig[1, 2] and the premature infant.[3] Sealock[1] has reported that normal human beings on a "diet practically free of ascorbic acid" excrete significant amounts of homogentisic acid when ingesting tyrosine, which was prevented by "reasonably large doses of crystalline ascorbic acid." However, no quantitative data are presented as to the amount of tyrosine ingested, the excretion of homogentisic acid, or the amount of ascorbic acid given.

In the present observations, the ingestion of tyrosine orally by scorbutic patients resulted in a markedly increased excretion of "tyrosyl" derivatives. The method used for the detection of the "tyrosyl" compounds theoretically measures tyrosine, *p*-hydroxyphenylpyruvic acid, and *p*-hydroxyphenyl lactic acid. As the urines were not fractionated or the compounds isolated, it is impossible to say which ones were proportionally responsible for the increased excretion of hydroxyphenyl compounds. Evidence that a large proportion was due to *p*-hydroxyphenylpyruvic acid is found in the marked increase of the reducing power of the urine. However, this reaction is not specific since another intermediary of tyrosine metabolism, namely homogentisic acid, will cause the reduction of phosphomolybdic acid. Although qualitative tests for homogentisic acid in these urines were negative, it conceivably could have been present and could have affected the quantitative reduction of phosphomolybdic acid, but could not be detected qualitatively. The absence of homogentisic acid is more in keeping with the animal studies of Painter and Silva.[3]

In the scorbutic patients, the increased power of the urine to reduce phosphomolybdic acid lagged behind the increased excretion of "tyrosyl" derivatives, the latter increasing immediately after the ingestion of tyrosine. Likewise, the response of the reducing substances to ascorbic acid in one patient (Case 1) was slower than that of the total hydroxyphenyl compounds. Despite the nonspecificity of the methods used, the changes noted in the scorbutic patients would seem to be the result of the tyrosine and ascorbic acid administration since the regimes were constant except for these two variables.

The excretion of "tyrosyl" derivatives by scorbutic patients who were maintained on a vitamin C-free diet did not differ appreciably from that of normal subjects when they were not receiving tyrosine. The metabolic abnormality was noted only when they were given exogenous tyrosine. Furthermore, the scorbutic patient (Case 3) who received only ascorbic acid and no tyrosine showed no statistically significant change in excretion of "tyrosyl" derivatives after the administration of ascorbic acid. Normal Subject 3 demonstrated that the dietary regime had no significant role in "tyrosyl" excretion.

The scorbutic patients demonstrated a progressive increase in capillary fragility while receiving tyrosine. While the death of the patient (Case 4) cannot be ascribed to a hemorrhagic lesion as a result of scurvy, nevertheless, all the vitamin C-deficient patients showed progression of the scorbutic skin lesions associated with increased weakness and lassitude during administration of *l*-tyrosine. Presumably the administration of amino acid in these large amounts placed an increased demand on the unmeasured body stores of vitamin C. In the original work of Sealock,[1] it is implied that the feeding of tyrosine results in a depletion or excess utilization of the ascorbic acid of the body. This phenomenon was apparently demonstrated clinically under the conditions used in the present study by the progressive intensity of the signs and symptoms of scurvy. However, in the normal individual, there was no significant decrease in the ascorbic acid content of the whole blood during the administration of tyrosine, and when these same individuals were given added ascorbic acid before and during administration of tyrosine, it did not change their response to this amino acid.

That the abnormality of the metabolism of hydroxyphenyl compounds has wider implications than in scurvy alone has support in the effect that liver extract and pteroylglutamic acid have on the metabolism of hydroxyphenyl compounds. Swendseid and co-workers[10, 11] have reported that patients with pernicious anemia excrete abnormally large amounts of hydroxyphenyl compounds and that the excretion of these compounds decreases to normal levels when liver extract is given. Sealock and Lepow[12] also have shown that antipernicious anemia extracts partially improved the tyrosine metabolism of scorbutic guinea pigs. Likewise, pteroylglutamic acid has been shown to have an effect on the metabolism of hydroxyphenyl compounds in scorbutic guinea pigs both in vivo[13] and in vitro experiments.[14] In addition, the administration of ascorbic acid to animals deficient in pteroylglutamic acid has altered the course of this deficiency.[15]

SUMMARY

The metabolism of tyrosine was studied in four patients with scurvy and in three normal individuals. In six of these individuals, 20 Gm. of tyrosine per day was given by mouth for periods varying from five to thirty-one days.

The patients with scurvy, while on a vitamin C-free diet and oral administration of tyrosine, excreted large amounts of "tyrosyl" derivatives, and the ability of their urine to reduce phosphomolybdic acid increased markedly, presumably due in part to the presence of *p*-hydroxyphenylpyruvic acid. The addition of ascorbic acid to this regime in the scorbutic patients resulted in a rapid decrease in the excretion of "tyrosyl" derivatives and the disappearance of the abnormal reducing material in the urine.

The excretion of "tyrosyl" derivatives in normal individuals on tyrosine was comparable with that of scorbutic subjects during the period when they were receiving ascorbic acid. At no time did the reducing substance of the urine of normal individuals rise significantly while they were receiving tyrosine. The addition of ascorbic acid to a normal individual's diet did not change the response to the administration of tyrosine.

It is concluded that individuals with scurvy have a marked defect in the metabolism of tyrosine and hydroxyphenyl compounds and that previous experiments with scorbutic animals and premature infants are in general agreement with the findings reported here.

It seems unlikely that this defective metabolism of tyrosine plays a significant role in the clinical picture of scurvy since the excretion of "tyrosyl" derivatives was not remarkable unless added tyrosine was given.

The clinical status of scurvy is made more severe during tyrosine therapy with increase in capillary fragility. These manifestations are completely controlled when ascorbic acid is added to the diet despite continued administration of tyrosine.

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OBSERVATIONS ON THE ETIOLOGIC RELATIONSHIP OF ACHYLIA GASTRICA TO PERNICIOUS ANEMIA

XI. Hematopoietic Activity in Pernicious Anemia of a Beef Muscle Extract Containing Food (Extrinsic) Factor Upon Intravenous Injection Without Contact With Gastric (Intrinsic) Factor

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PREVIOUS observations[1, 2] have shown that 200 Gm. of beef muscle are hematopoietically active in addisonian pernicious anemia when 150 ml. of normal human gastric juice also are given by mouth within six hours, or preferably simultaneously, as a daily regime for a period of ten days. If an acid mixture of beef muscle and gastric juice is incubated for twelve hours for the purpose of effecting peptic digestion of the beef muscle and is then neutralized, it also is active.[3] On the other hand, if the incubated mixture is heated to 100° C. for five minutes, its hematopoietic activity as determined by oral administration is destroyed,[3] whereas that of whole liver or liver extract is not detectably affected by such a procedure. From this it was assumed that the thermostable antipernicious anemia principle of liver was *not* formed by the incubation procedure in vitro and that the effect of heat was merely to destroy a thermolabile factor in the gastric juice.

If a beef muscle and gastric juice mixture is given at an acid pH (1.8 to 3.5) subsequent to the twelve-hour acid incubation period, no hematopoietic effect appears.[3] If, however, this acid incubated mixture is treated with alkali to give a pH of 5 to 7 just prior to oral administration, it is active. This suggested that a preliminary chemical interaction occurred between the so-called extrinsic factor of beef muscle and the intrinsic factor of normal human gastric juice at or about neutrality within the intestinal tract. Such a reaction was conceived as necessary for the eventual formation of the antipernicious anemia principle in the body. It seemed probable, therefore, that the extrinsic factor was chemically different from the antipernicious anemia principle of liver.

Because of this evidence, the increased hematopoietic activity in pernicious anemia of liver[4] and of relatively crude liver extracts[5] when given orally together with normal human gastric juice was originally assumed to indicate the presence of extrinsic factor as well as of the antipernicious anemia principle.[6] However, it was later shown[7] that even refined liver extracts when given by

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mouth were potentiated in their hematopoietic effect by gastric juice and that destruction of the antipernicious anemia principle of such extracts by hydrolysis with 5 per cent sulfuric acid resulted likewise in the loss of extrinsic factor activity. Recently it was found[7] that the hematopoietic activity of orally administered pure vitamin B_{12} derived from liver (presumably the antipernicious anemia principle) also was enhanced by the simultaneous administration of normal human gastric juice—but that the activity of the combination when given orally was not so great as that of the vitamin B_{12} when administered alone parenterally.

The observations reported here concern questions obviously raised by these findings: (1) Can extrinsic factor *alone* (beef muscle) act directly as the antipernicious anemia principle; that is, is a suitable preparation of beef muscle, like vitamin B_{12}, hematopoietically effective in pernicious anemia upon parenteral administration without contact with gastric juice? (2) Is the hematopoietic potentiating action of intrinsic factor in pernicious anemia *specific* only for vitamin B_{12} and chemically similar substances in beef muscle and in other foods; or does intrinsic factor also facilitate the nonspecific absorption or otherwise enhance the hematopoietic action of other substances?

METHODS

All patients studied had addisonian pernicious anemia either in relapse or without previous treatment. The diagnosis was established by the characteristic blood and bone marrow morphology and by the presence of histamine-fast achlorhydria. It was confirmed in each patient by one or more reticulocyte responses to specific therapy and eventually by a return to normal blood values as a result of liver extract therapy. During the observations the basal diet of the patients contained no meat, fish, or eggs, and little milk. The administration of the test substances, including the normal human gastric juice, was made at 8 P.M. The last meal of the day, consisting of tea and white toast, was at 4 P.M. This separated the test substance by at least four hours from any food constituent considered likely to contain extrinsic factor.

The attempted plan of the observations was to administer to the patients during three consecutive periods of ten or more days each a uniform daily dose of extrinsic factor derived from 400 Gm. of beef muscle first orally, then orally with 150 ml. of normal human gastric juice, and finally intravenously *without* gastric juice. In evaluating the hematopoietic activity of the various preparations used, the method of serial reticulocyte responses[8] was employed.

Formijne[9] has shown that a 70 per cent alcohol extract of beef muscle possesses extrinsic factor activity in pernicious anemia. Consequently the preparation of such an extract was undertaken, starting with a concentrated aqueous extract of lean beef muscle,* of which 1 Gm. was derived from 14 to 15 Gm. of beef muscle. Following dilution of the aqueous extract with an equal volume of distilled water, a 70 per cent alcohol concentration by volume was made by the addition of suitable amounts of 95 per cent ethyl alcohol. The

*Kindly supplied by Dr. William Peabody of the Valentine Company, Inc., Richmond, Va.

TABLE I. EFFECT OF DAILY ADMINI

	10 ML. MEAT EXTRACT* ORALLY						10 ML. MEAT EXTRACT* AND 150 ML. GASTRIC JUICE ORALLY							
CASE	109		110		111		105		109		110		111	
PERIOD	I		I		I		I		II		II		II	
DAY OF THERAPY	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)
0	1.28	0.4	1.72	1.3	1.82	0.7	1.87	1.5	1.66	0.8		1.9		1.0
1		0.4		0.7		0.9		1.3		0.6		1.3		1.4
2		0.6		1.0	1.75	0.7	1.83	1.3		0.3		1.5		1.9
3		0.3	1.73	0.9		1.3		1.4	1.62	0.5	2.15	1.6	1.42	1.5
4	1.28	0.6		1.1		1.4	1.78	1.2		1.4		1.1		1.9
5	Tsf.	0.7		1.4	1.54	1.2		1.3		1.3		1.3		2.6
6		0.7	1.96	1.9		0.8	1.91	2.3	1.35	1.4	1.85	2.0	1.40	2.5
7	1.59	0.6		**3.4**		0.9		3.8		1.0		**2.5**		3.2
8		0.7		2.1	1.58	0.8	1.90	5.2		1.2		1.7		3.7
9		1.2	2.24	2.6		1.0		5.9	1.59	1.6	2.53	1.7	1.52	4.5
10	1.66	0.8		1.9	Cont'd 111-II		2.11	6.8		2.9		1.7		4.1
11		†	Cont'd 110-II					7.2		2.3	Cont'd 110-III	1.5		4.8
12	Cont'd 109-II						2.49	3.7	1.50	1.5				3.5
13								5.3		1.8			1.54	2.2
14							2.58	2.3		2.3				2.2
15							End		1.40	3.3			Cont'd 111-III	
16										5.0				
17										8.0				
18									1.44	7.2				
19										7.4				
20										**10.9**				
21									1.99	7.9				
22										8.3				
23										9.1				
24									2.10	10.1				
25										7.1				
26										3.1				
									Cont'd 109-III					

Tsf., Transfusion of 250 ml. of packed red blood cells.

*10 ml. of meat extract are derived from 400 Gm. of lean beef muscle.

†Rule indicates end of therapy in period.

flocculent precipitate resulting was allowed to settle overnight at 5° C. The supernatant liquid was then filtered through a fluted paper filter and the precipitate washed on the paper with one-fifth the initial volume of 70 per cent alcohol. The precipitate was then discarded. Thereafter, the alcoholic filtrate was concentrated by free evaporation on electric hot plates. At the final point of concentration, the boiling point reached 108° ± 4° C. The volume of the concentrated filtrate was then so adjusted with distilled water that 1 ml. was derived from 40 Gm. of the original beef muscle. The filtrate was then bottled and autoclaved for twenty minutes. Throughout the preparation of the alcoholic extract, precautions were maintained to employ pyrogen-free fluids and apparatus.

When the preparation was given orally, 10 ml. were diluted in either 150 ml. of physiologic saline or in 150 ml. of neutralized normal human gastric juice secreted after the injection of histamine. When given intravenously, 10 ml. of the extract were mixed with 500 ml. of 5 per cent sterile dextrose and water. The slow intravenous administration of the meat extract was followed in a few instances by moderate or mild febrile reactions. These reactions

ADMINISTRATION OF VARIOUS SUBSTANCES

10 ML. MEAT EXTRACT* INTRAVENOUSLY												1 μG VITAMIN B_{12} INTRAMUSCULARLY			
106		107		108		109		110		111		108		111	
I		I		I		III		III		III		II		IV	
R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)	(MIL.) R.B.C.	RET. (%)	R.B.C. (MIL.)	RET. (%)	R.B.C. (MIL.)	RET. (%)
2.18	0.2	2.07	0.9	1.79	0.3	2.16	2.0		1.5		2.2	1.55	5.6	1.79	1.2
	0.4		0.8		0.2		4.2	2.32	2.1		1.7		5.0		1.8
2.12	0.0		0.9	1.51	0.3		5.0		—	1.41	1.6		4.3		2.8
	0.0		0.8		0.2	2.15	2.9		1.3		1.2	1.67	3.1	2.23	3.2
2.39	0.1		1.2		0.4		1.2	2.39	1.6		2.2		3.2		4.1
	0.4	2.17	2.0	1.44	0.6		1.8		2.5		2.6		4.2		4.9
2.21	1.8		2.9		0.4	2.30	2.5		3.3	1.51	4.6	1.80	5.1	2.48	7.6
	4.2		3.1		0.6		4.4	2.58	3.9		5.5		6.3		4.6
2.23	7.3		4.9		0.8		6.4		3.7		6.7		7.3		5.6
	8.3		4.6	1.51	1.3	2.63	4.8		2.6	1.60	5.9	2.00	5.3	2.58	4.8
2.49	9.3		3.6		2.1		5.4	2.65	1.1		5.6		4.8		4.7
	6.4		2.5		2.8		2.7	End			7.8		3.9		3.9
2.25	3.3	2.69	3.1		3.6	3.23	1.2			1.61	7.8		5.8	2.48	3.7
	2.6		3.8		4.7	End					6.3	2.13	3.6		2.0
2.58	2.6		2.4	1.39	5.4						7.3		5.3	End	
End			1.8		5.9					1.63	7.1		5.3		
		3.01	1.3		5.7						6.0	2.31	5.2		
			1.6	1.55	5.6						5.4	End			
			1.6	Cont'd 108-II				0.5 μg of vitamin B_{12} daily		1.75	4.2				
		3.09	2.0								3.9				
		End								1.74	3.4				
											2.1				
											1.4				
										1.79	1.2				
										Cont'd 111-IV					

usually developed with the first two or three injections and did not thereafter recur. No signs of subsequent sensitization to the extract or to beef appeared in any of the patients.

OBSERVATIONS

Is Extrinsic Factor Hematopoietically Effective Upon Parenteral Administration Without Intrinsic Factor? The results of the observations upon the hematopoietic effect of the 70 per cent alcohol extract of beef muscle in seven patients with pernicious anemia are shown in Table I. Although the individual reticulocyte peaks are small, the trend of their values and the time of their maxima and of the clinical improvement exhibited by the patients convinced the observers of the validity of the conclusions drawn under the usual carefully controlled conditions of the tests. In one patient, Case 105, the extract derived from 600 Gm. of beef muscle given orally daily together with 150 ml. of normal human gastric juice was hematopoietically active. In two patients, Cases 109 and 111, the extract derived from 400 Gm. of beef muscle was inert upon daily oral administration, was slightly active upon daily oral administration with 150 ml. of normal human gastric juice, and was still more active upon daily intravenous administration without gastric juice. In one patient, Case 110, the preparation of extrinsic factor was weakly active upon

daily oral administration, was slightly more active when given with normal human gastric juice, and was still more active upon intravenous administration without gastric juice. Three patients, Cases 106, 107, and 108, received the extract without gastric juice by intravenous injection only. A definite reticulocyte response appeared in each instance. In Case 107 the red blood cell count

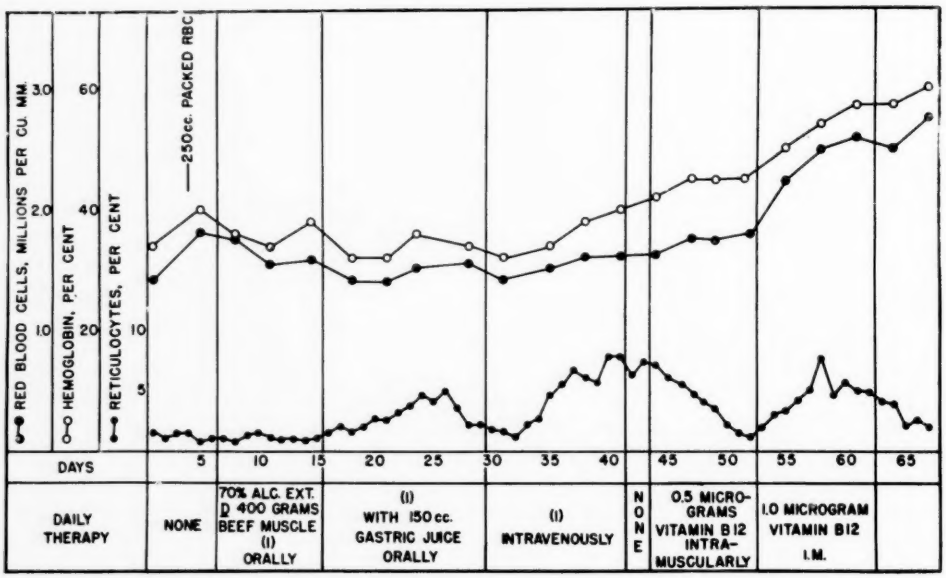

Fig. 1.—Hematopoietic effects in Case 111 of the uniform daily administration of various substances in successive periods as shown. (*I*) refers to 10 ml. of 70 per cent alcohol soluble meat extract derived from 400 Gm. of beef muscle. Note the three successive reticulocyte peaks indicating, respectively, activation of the extract upon oral administration with normal human gastric juice, its greater activity upon intravenous administration without gastric juice, and finally the still greater activity of vitamin B_{12} when given in a dose of 1 μg daily.

increased in twenty days from 2.07 to 3.09 millions per cubic millimeter. In two patients, Cases 108 and 111, the daily intramuscular administration of 1 μg of crystalline vitamin B_{12}* following the period of intravenous administration of the beef muscle extract resulted in another definite reticulocyte peak. From this it was inferred that the hematopoietic activity of the extrinsic factor preparation upon parenteral administration was distinctly less than that of 1 μg of vitamin B_{12}. The results in Case 111 are shown in Fig. 1.

Is the Hematopoietic Potentiating Action of Intrinsic Factor Specific? In order to determine whether or not normal human gastric juice possesses a nonspecific effect in enhancing absorption from the alimentary tract in pernicious anemia, the blood levels of test substances presumably unrelated to vitamin B_{12} were followed after oral administration to seven patients with pernicious anemia. Most of the observations were made before treatment, but a few were conducted subsequently during remission after specific therapy. Thus, blood glucose and blood tyrosine levels were determined following the

*Kindly supplied by Dr. Augustus Gibson of Merck & Co., Inc., Rahway, N. J.

oral administration of single doses of 50 and 10 Gm. respectively of those substances to fasting patients. The test substances were mixed together and given simultaneously with either 150 ml. of normal human gastric juice or with an equal amount of physiologic salt solution upon alternate days. Blood alpha-amino nitrogen and nonprotein nitrogen levels were similarly followed after the ingestion of 50 Gm. of vitamin-free casein (Labco) (previously shown to contain no extrinsic factor)[10] with either physiologic saline or normal human gastric juice upon different days. Glucose determinations were made according to the method of Folin and Wu[11] and of Folin,[12] tyrosine by the method of Bernhart and Schneider,[13] alpha-amino nitrogen by the method of Krauel,[14] and nonprotein nitrogen by the method of Folin and Wu.[15] As shown in Table II, no consistently enhanced absorptive effects upon glucose, *l*-tyrosine, or casein were observed that could be attributed to the gastric juice in any of the patients with pernicious anemia so tested, before effective therapy. Not shown are the similar negative results obtained in a few patients after treatment.

The possible hematopoietic potentiating effect of normal human gastric juice upon synthetic pteroylglutamic acid,* either by increasing its absorption or by other action, was also assayed in two patients, Cases 112 and 113, not included in the tables. To each, 0.5 mg. of pteroylglutamic acid was given orally daily during a first period of ten or more days together with 150 ml. of physiologic saline, and during a second period of ten days the same amount of pteroylglutamic acid was given orally daily together with 150 ml. of normal human gastric juice. In the first period of Case 112 a reticulocyte peak of 9.3 per cent was reached on the eighth day following an initial red blood cell count of 1.63 million per cubic millimeter. In Case 113 the corresponding value was 8.1 per cent reticulocytes on the eleventh day following an initial red blood cell level of 1.86 million per cubic millimeter. When gastric juice replaced the physiologic saline in the second periods no detectable reticulocyte peaks appeared in either patient. Thus, no hematopoietic potentiating effect of normal human gastric juice upon pteroylglutamic acid was observed.

DISCUSSION

In 1938 Dock[16] wrote, "It seems probable that beef muscle contains the same material as liver, but in quantities too small to be effective unless absorption is increased by gastric or enteric ferment. For some reason, parenteral administration of muscle extract seems not to have been tried, but extract of muscle which had been incubated with gastric juice was potent in Wilkinson's experiments."

The potentiation of the hematopoietic activity of vitamin B_{12} with gastric juice upon oral administration[7, 17] and the present confirmation of our preliminary observation[7] that the extrinsic factor of beef muscle is hematopoietically active upon parenteral administration *without gastric juice* go far to support Dock's surmise. It is of interest that Wilkinson[18] employed by daily intramuscular injection an extract derived from about the same amount of

*Folvite: kindly supplied by Dr. Guy W. Clark of Lederle Laboratories, Inc., Pearl River, N. Y.

TABLE II. NEGATIVE EFFECT OF NORMAL HUMAN GASTRIC JUICE IN AUGMENTING THE ABSORPTION OF ORALLY ADMINISTERED GLUCOSE, L-TYROSINE, OR DIGESTION PRODUCTS OF VITAMIN-FREE CASEIN

CASE	DATE	FOLLOWING 50 GM. OF GLUCOSE BY MOUTH — BLOOD GLUCOSE (MG. PER 100 ML.)					VEHICLE (150 ML.)	FOLLOWING 10 GM. OF L-TYROSINE BY MOUTH — BLOOD TYROSINE (MG. PER 100 ML.)				
		FASTING	1 HR.	2 HR.	3 HR.	4 HR.		FASTING	1 HR.	2 HR.	3 HR.	4 HR.
107	2/ 7/49	87	163	174	117	89	Saline*	1.2	5.8	8.6	11.2	11.8
	2/ 8/49	117	248	271	186	125	N.H.G.J.†	1.1	4.2	6.1	5.1	6.7
	2/ 9/49	77	170	167	117	78	Saline					
	2/11/49	86	191	164	96	106	N.H.G.J.	1.6	8.1	9.6	8.7	8.5
108	12/15/48	131	133	140	131	120	Saline	1.3	3.2	3.5	4.5	4.2
	12/16/48	110	134	135	114	92	N.H.G.J.	2.1	4.7	6.9	7.4	5.0
	12/17/48	101	135	132	117	101	Saline	2.5	4.3	4.6	6.1	5.9
	12/20/48	125	118	143	144	134	N.H.G.J.	1.6	3.4	-	4.6	7.1
109	3/ 1/49	85	-	88	107	87	Saline	1.4	2.1	2.6	3.5	4.5
	3/ 3/49	101	102	133	111	87	N.H.G.J.	1.4	2.6	3.8	4.3	5.3
110	3/ 1/49	64	85	86	66	63	Saline	1.2	2.9	4.4	4.6	4.5
	3/ 3/49	96	106	116	94	79	N.H.G.J.	1.5	2.9	3.9	4.7	4.7
111	2/ 2/49	114	138	143	133	136	Saline	1.1	3.0	4.4	5.2	4.2
	2/ 5/49	96	144	138	113	90	N.H.G.J.	1.4	3.0	4.0	4.1	3.2
	2/11/49	107	101	113	81	93	N.H.G.J.	1.5	4.4	6.5	6.4	4.1
112	3/23/49	80	150	122	92	86	Saline	1.2	2.7	3.4	3.3	4.3
	3/28/49	-	88	145	126	75	N.H.G.J.	1.1	3.2	4.0	6.6	6.8

CASE	DATE	FOLLOWING 50 GM. OF VITAMIN-FREE CASEIN BY MOUTH — BLOOD NONPROTEIN NITROGEN (MG. PER 100 ML.)					VEHICLE (150 ML.)	BLOOD ALPHA-AMINO NITROGEN (MG. PER 100 ML.)				
		FASTING	1 HR.	2 HR.	3 HR.	4 HR.		FASTING	1 HR.	2 HR.	3 HR.	4 HR.
109	2/26/49	27.6	32.0	33.6	32.8	35.2	Saline	5.7	6.6	7.7	8.1	7.6
	2/25/49	26.8	24.4	34.4	33.2	37.2	N.H.G.J.	6.7	6.7	7.4	7.7	8.0

*0.85 per cent solution of NaCl.
†Normal human gastric juice.

beef muscle as we used. Although it was administered subsequent to incubation with an extract of hog mucosa, it seems probable that this was the first demonstration, though not then so interpreted, of the hematopoietic activity of a beef muscle extract upon parenteral administration.

The potentiating effect of normal human gastric juice upon orally administered vitamin B_{12} has been demonstrated.[7, 17] The present observations fail to show any enhanced absorption by gastric juice of *l*-tyrosine, glucose, or of the digestive products of orally administered casein. Pteroylglutamic acid was not demonstrably more active in producing a reticulocyte response when given with gastric juice than with an equal amount of physiologic salt solution. On the basis of the present evidence, then, the action of intrinsic factor appears to be a specific one—presumably a hematopoietic potentiating effect on substances possessing identity with or chemical similarity to vitamin B_{12}.

There is suggestive evidence, however, that vitamin B_{12} may exist in nature, not necessarily in the pure form employed in these observations, but rather as protein "conjugates" or analogues. Thus, Smith and Parker[19] noted the "dramatic" increase in concentration effected by hydrolysis with trypsin of liver fractions containing vitamin B_{12}. According to Cohn and associates,[20] the antipernicious anemia activity of liver may remain largely with the proteins if their denaturation is avoided during the chemical fractionation of the liver. A synthetic modification of vitamin B_{12} known as vitamin B_{12a}[21] with definite though reduced hematopoietic activity has recently been made by hydrogenation of vitamin B_{12}. By chromatography a second crystalline variant, designated as vitamin B_{12b}, has been prepared from liver and appears to possess biologic activity in both chick and microbial assays.[22]

Finally, from the comparative degrees of potentiation of pure vitamin B_{12} and of the present beef muscle extract by normal human gastric juice, at least an impression of a discrepancy is conveyed. Thus, in our previously reported observations,[7] with the exception of Case 102, the potentiation of the activity of 5 μg of pure vitamin B_{12} by gastric juice, though definite, was not remarkable in terms of reticulocyte responses. Yet in the present observations upon Cases 108 and 111, the daily dose, 10 ml., of the beef muscle extract employed, although possessing upon intravenous injection distinctly less than the hematopoietic activity of 1 μg of pure vitamin B_{12}, was nevertheless capable of detectable increase in hematopoietic effect when given orally in the same dosage with gastric juice. According to microbial assays using the organism *Lactobacillus lactis* Dorner,* the vitamin B_{12} content of 10 ml. of this beef muscle extract was 0.9 microgram. Assay of the same extract using the organism *Lactobacillus leichmannii*[23]† demonstrated 0.37 μg of vitamin B_{12} per 10 milliliters. In the latter method the effect of bacterial growth promoting substances other than vitamin B_{12} is stated to be determined by difference following destruction of the vitamin B_{12} in an aliquot portion of the sample by alkaline hydrolysis. This procedure completely destroyed the microbial activity for

*These assays were kindly performed by the laboratories of Merck & Co., Inc., Rahway, N. J.

†These assays were kindly performed by Dr. Thomas H. Jukes of Lederle Laboratories, Inc., Pearl River, N. Y.

L. leichmannii, and so presumably indicates the absence of significant quantities of thymidine or of other desoxyribosides. The results obtained upon microbial assay are seemingly in reasonably close agreement with each other and with the clinical effects upon intravenous injection in Cases 108 and 111. However, as indicated, the potentiating effect of gastric juice upon the beef muscle extract when orally administered is perhaps greater than would be expected from less than 1 μg of vitamin B_{12}. Thus, it is possible that substances in the beef muscle extract other than microbially active vitamin B_{12} may be effective as food (extrinsic) factor in pernicious anemia. Yet, so far as can be inferred from the negative results of the studies with glucose, *l*-tyrosine, casein, and pteroylglutamic acid, the function of the gastric (intrinsic) factor is a specific one.

SUMMARY AND CONCLUSIONS

1. Observations were made during successive periods of ten or more days in seven patients with addisonian pernicious anemia employing a 70 per cent alcohol extract of beef muscle as a source of food (extrinsic) factor.

2. When 10 ml. of the extract derived from 400 Gm. of beef muscle were given daily by mouth to these patients, a detectable reticulocyte response appeared in only one instance. When the extract was given together with 150 ml. of normal human gastric juice to four patients, reticulocyte responses appeared in all instances, and when the material was subsequently given intravenously without gastric juice to three of these patients, another reticulocyte response appeared, thus indicating greater hematopoietic activity.

3. In two patients it was shown that the hematopoietic effect of 10 ml. of the beef muscle extract upon daily intravenous injection was less than that of the daily intramuscular injection of 1 μg of crystalline vitamin B_{12}. Microbial assays indicated that 10 ml. of the beef muscle extract contained from 0.37 to 0.9 μg of vitamin B_{12} activity.

4. However, judging from our previous observations upon the potentiation of 5 μg of crystalline vitamin B_{12} by normal human gastric juice, the hematopoietic activity of the beef muscle extract when given orally with gastric juice appeared to be surprisingly great.

5. Only this fact suggests that substances in the meat extract other than vitamin B_{12} are susceptible of potentiation by normal human gastric juice upon oral administration in pernicious anemia.

6. No evidence was obtained for a nonspecific effect of gastric (intrinsic) factor in increasing the hematopoietic action of pteroylglutamic acid or in promoting the intestinal absorption of glucose, *l*-tyrosine, or the digestion products of casein.

We desire to express our indebtedness to the late Dr. Lionel Berk, whose death unhappily interrupted the preliminary work that led to the present observations. We have received much valuable advice from Dr. Arnold B. Welch and Dr. Robert W. Heinle of the School of Medicine of Western Reserve University. Finally, we are grateful to Miss Phyllis Gordon, Mrs. Katharine Bingham, and Miss Angela Pasquariello for the performance of the blood examinations.

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FURTHER OBSERVATIONS ON THE USE OF THE URINARY PIGMENT-CREATININE RATIO FOR THE MEASUREMENT OF BASAL METABOLIC RATE

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IN A previous communication,* it was found that an accurate correlation existed between the basal metabolic rates as determined by the respiratory calorimeter and the urinary pigment-creatinine ratio (P/C).† On the basis of statistically valid results obtained in 156 adult female subjects and 57 adult male subjects, regression equations were derived which permit the calculation of the basal metabolic rate from the urinary pigment-creatinine ratio. These equations are:

Male	B.M.R. = 57.0 + 0.25 P/C
Female	B.M.R. = 54.7 + 0.22 P/C

This present study is an analysis of the results obtained in comparing the basal metabolic rates determined by the respiratory calorimeter and the pigment-creatinine ratio in 740 observations made on 424 adult patients. Of the 740 observations, 457 (61.7 per cent) were on euthyroid patients, 168 (22.8 per cent) were on hyperthyroid patients, and 115 (15.5 per cent) were on hypothyroid patients. The clinical material consisted of hospital, private, and out-patient department patients.

RESULTS

We have previously reported* that the determination of the pigment-creatinine ratio is unreliable in the presence of increased bilirubinemia, azotemia, and in the presence of hemoglobinuria and drugs and foods which impart an abnormal color to the urine (e.g., riboflavin, aureomycin, beets, and rhubarb). There were 32 such cases in our series. The pigment-creatinine determination is also deficient in measuring hypometabolism, rarely indicating metabolic rates of less than –15 per cent. There were 115 observations on cases of this type in our series. Thus, there were 147 instances in which the pigment-creatinine determination would not be expected to be of diagnostic value.

Of the remaining 593 observations, the B.M.R. as determined by the respiratory calorimeter and the pigment-creatinine ratio agreed with each other and with the clinical findings in 508 cases (85.6 per cent). Of these 508 observations, 380 (64.0 per cent) were on euthyroid patients and 128 (21.6 per cent) were on hyperthyroid patients. In 58 cases (9.8 per cent) the B.M.R. as determined by the pigment-creatinine ratio was more consistent with the clinical findings than the B.M.R. determined on the basis of oxygen con-

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†The chemical determinations involved in this test can be done in about twenty-five minutes and can be performed easily in any routine chemical laboratory.

TABLE I. RESULTS OF TESTS—EXCLUDING OBSERVATIONS IN WHICH P/C WOULD NOT BE USED FOR DIAGNOSTIC PURPOSES*

RESULT	NUMBER OF OBSERVATIONS		PER CENT OF TOTAL	
B.M.R. and P/C agree with each other and with the clinical findings				
a. Euthyroids	380		64.0	
b. Hyperthyroids	128		21.6	
		508		85.6
P/C more accurate than B.M.R.				
a. Euthyroids	48		8.1	
b. Hyperthyroids	10		1.7	
		58		9.8
B.M.R. more accurate than P/C				
a. Euthyroids	14		2.4	
b. Hyperthyroids	13		2.2	
		27		4.6
Total		593		100.0

*The P/C determination is not reliable in cases of jaundice, azotemia, and in the presence of hemoglobinuria or drugs that color the urine (32 cases); or in hypothyroidism—metabolism below −15 per cent (115 cases).

sumption. Of these 58 cases, 48 (8.1 per cent) were euthyroid patients and 10 (1.7 per cent) were hyperthyroid patients. In 27 cases (4.6 per cent) the B.M.R. determined by the pigment-creatinine ratio was less in agreement with the clinical findings than was the B.M.R. as determined by the respiratory calorimeter. Of these 27 cases, 14 (2.4 per cent) were euthyroid patients and 13 (2.2 per cent) were hyperthyroid patients. Thus, in 566 observations (95.4 per cent), the B.M.R. as determined by the pigment-creatinine ratio in normal and hyperthyroid patients was of diagnostic value. In the remaining 27 cases (4.6 per cent) the pigment-creatinine metabolic rate was inaccurate.

DISCUSSION

The observation that the B.M.R. as calculated from the urinary pigment-creatinine ratio is at least as accurate as that calculated from the respiratory calorimeter in 95.4 per cent of instances when cases in which the pigment-creatinine ratio would not be expected to be accurate are eliminated indicates that the pigment-creatinine B.M.R. should be used to check the oxygen consumption B.M.R. when the latter does not seem to agree with the clinical picture. In addition, there is a group (58 cases or 9.8 per cent of patients having metabolism tests) in which the B.M.R. calculated by the pigment-creatinine ratio should be used in preference to the B.M.R. determined by oxygen consumption. We were able to break down this group into five types:

1. Cases of hyperthyroidism under prolonged therapy in which the B.M.R. remains elevated in spite of clinical improvement—10 cases.

Example: A. E., a 48-year-old white man, was seen in the Outpatient department for the first time in December, 1945, complaining of weakness, nervousness, palpitations, and weight loss for one year. Physical examination revealed an enlarged firm thyroid gland, warm moist skin, and a tachycardia. B.M.R. was +50. He was given thiouracil and later changed to propylthiouracil in adequate dosage. The patient showed gain in weight and disappearance of all signs of thyrotoxicosis except that repeated B.M.R. determinations remained between +30 and +48. The patient volunteered this information, "When taking

a B.M.R. I'm always nervous and feel as if I'm choking.'' In July, 1948, it was decided to stop all antithyroid medication as the patient was asymptomatic although the B.M.R. was +30. In November, 1948, while the patient was still clinically euthyroid, the oxygen consumption B.M.R. was +32 while the B.M.R. calculated on the basis of urinary pigment-creatinine ratio was successively −4 and +2. In January, 1949, while still asymptomatic, the oxygen consumption B.M.R. was +36 while the B.M.R. calculated from the P/C was −8.

2. Cases in which an anxiety state or neurocirculatory asthenia causes an abnormally elevated B.M.R. inconsistent with the clinical findings—32 cases.

Example A: S. C., a 47-year-old white woman, was admitted to the hospital March 3, 1949. She gave a three-year history of nervousness, hypertension, palpitations, heat intolerance, and sweating. In December, 1946, her B.M.R. was +50 and a subtotal thyroidectomy was performed. Her symptoms persisted for eighteen months postoperatively. In July, 1948, she had a B.M.R. of +7 with all the symptoms of thyrotoxicosis still present. Her present admission was due to the appearance of a small nodule on the left side of the thyroid gland. She showed no evidence of weight loss; there was no tremor; the pulse rate was 100. She was quite apprehensive about the prospect of further surgery. On March 9, 1949, the oxygen consumption B.M.R. was +24 whereas the P/C B.M.R. was −11. On March 15, the oxygen consumption B.M.R. was +21 while the P/C B.M.R. was −13. On March 16, the patient was reassured that no surgery would be necessary. She slept well that night and on the following day the oxygen consumption B.M.R. was +7 and the P/C B.M.R. was −8. The patient was discharged from the hospital with the diagnosis of menopause, essential hypertension, and anxiety state. This patient had received no specific therapy at any time during her hospital stay.

Example B: G. A., a 27-year-old white woman, was admitted to the hospital in January, 1949, for an evaluation of an asymptomatic hypertension known to be present for two months. The only significant finding on physical examination was a blood pressure of 200/140. On January 19, a B.M.R. was reported as +34; the P/C B.M.R. calculation was −7. On January 25, a repeat oxygen consumption B.M.R. was −1; the P/C B.M.R. at this time was −2. There was no specific therapy given during the hospital stay. The patient was discharged with a diagnosis of essential hypertension.

Example C: E. W., a 30-year-old white woman, was referred to the private office of one of the authors (J. J. V.) for the first time in April, 1949, because she had been found to have a B.M.R. of +60 two weeks previously. She complained of palpitations and a 22 lb. weight loss in the past twenty months following a pregnancy. Physical examination revealed a warm moist skin, thyroid moderately enlarged, pulse rate 128 per minute, and slight prominence of the eyes. A B.M.R. at this time was +47. The patient was started on cyclohexylmethylthiouracil, 300 mg. per day. With the institution of therapy, the patient began to feel better and gain weight, and the pulse rate fell although the B.M.R. remained elevated. Five weeks after she was first seen, she had no complaints, had gained 14¼ lb., and the pulse rate was 72 per minute. A B.M.R. at this time was +33. Because of the discrepancy between the clinical condition of the patient and the oxygen consumption B.M.R., a P/C B.M.R. was determined and found to be −3.

3. Cases in which it is not technically possible to obtain an accurate B.M.R. (e.g. edentulous patients, patients with punctured eardrums, air swallowers)—5 cases.

Example: G. S., a 67-year-old white woman, complained of nervousness. There was no evidence of hyperthyroidism clinically, but because the patient was edentulous, numerous attempts to do a basal metabolism test were unsuccessful. In October, 1948, two successive P/C B.M.R. determinations were +3 and −2.

4. Cases in which a basal metabolism test is done on a patient for the first time as part of a routine work-up and yields a high result where there is no evidence of hyperthyroidism—10 cases.

Example: S. F., a 56-year-old white woman, came to the Outpatient department complaining of pains in the knees, which were diagnosed as due to osteoarthritis. She was 58 inches tall and weighed 202 pounds. Aside from the obesity and arthritis, the physical examination was negative. A routine oxygen consumption B.M.R. was +30. The P/C B.M.R. was +8.

5. Cases in which the patient refuses to undergo a metabolism determination—1 case.

Example: I. C., a 22-year-old white woman, was known to be a diabetic for four years. She had had numerous hypoglycemic reactions. She was hospitalized because of frequent episodes of nervousness and sweating which her physician attributed to an anxiety state. She became very upset at the prospect of undergoing a basal metabolism test and refused it. A B.M.R. calculated on the urinary pigment-creatinine ratio was −3. This result confirmed her physician's impression.

SUMMARY

The results of the comparison of the basal metabolic rate as determined by the oxygen consumption method and by the urinary pigment-creatinine ratio method (P/C) in 740 observations made on 424 patients are reported. Of the 740 observations, 457 (61.7 per cent) were on euthyroid patients, 168 (22.8 per cent) were on hyperthyroid patients, and 115 (15.5 per cent) were on hypothyroid patients. The clinical material consisted of hospital, private, and out-patient department patients.

The B.M.R. as determined by the pigment-creatinine ratio (P/C) is unreliable in cases of hypothyroidism, hyperbilirubinemia, azotemia, and in cases in which the urine is discolored by drugs or foods. There were 147 such cases in this series. If these observations are eliminated, then the P/C B.M.R. is at least as accurate as the oxygen consumption B.M.R. in 95.4 per cent of the remaining cases.

The P/C B.M.R. should be used to check all cases in which the oxygen consumption B.M.R. does not agree with the clinical findings in euthyroid and hyperthyroid patients.

The P/C B.M.R. determination should be used in preference to the oxygen consumption B.M.R. for the following groups of patients (9.8 per cent of our series).

1. Cases of hyperthyroidism under prolonged therapy in which the B.M.R. remains elevated in spite of clinical improvement.

2. Cases in which anxiety state or neurocirculatory asthenia causes an abnormally elevated B.M.R. inconsistent with the clinical diagnosis.

3. Cases in which it is technically impossible to obtain an accurate B.M.R.

4. Cases in which a basal metabolism test is done on a patient for the first time and yields a high result where there is no evidence of hyperthyroidism.

5. Cases in which the patient refuses to undergo a metabolism determination.

The authors appreciate the technical assistance rendered by Mrs. Jennie Shatton, Miss Edna Arzt, and Miss Virginia Rechnitzer, and are grateful to Mr. Jules Joskow, Department of Economics, The City College of New York, for his statistical analysis of the results.

EFFECT OF RETAINED BRONCHIAL LIPIODOL ON BLOOD IODINE

LeRoy Hyde, M.D., Van Nuys, Calif., and Bernard Hyde, M.D., Los Angeles, Calif.

NUMEROUS studies have been made on the metabolism of iodine and blood iodine levels in normal people and in various pathologic conditions. Normal standards for protein-bound blood iodine have been established,[1, 2, 3] 3.0 to 8.5 μg per 100 ml. of blood being the quoted normal range. In our laboratory, 5 to 7 μg per 100 ml. is considered normal. Patients with pulmonary tuberculosis have normal protein-bound blood iodine values.[4] Normal blood iodine values have been secured in numerous other pathologic conditions. Most observers agree that hyperthyroidism produces an elevation of the protein-bound blood iodine.[1, 2, 5-8] Turner[2] found elevated blood iodine levels, up to 18 μg per 100 ml. of blood, in lymphatic leucemia. Several observers[1, 6] have found increased protein-bound blood iodine values in patients with liver and gall bladder disease. The effects of previously administered iodine-containing compounds such as gall bladder dyes must be always borne in mind in these latter patients. It is known that the liver takes up iodine and excretes it into the bile. Cohn and Feldman[5] quote Greene and Bruger as finding normal blood iodine values in liver and gall bladder disease, if no iodine-containing compounds had been taken previously. Cohn and Feldman[5] found experimentally that common bile duct ligation in cats failed to produce an elevation of blood iodine. The administration of gall bladder dyes, however, elevates the protein-bound blood iodine for several weeks. A group of patients with advanced liver disease was compared with a group of normal subjects, and blood iodine levels were found to be similar. Rabbits had no elevation of blood iodine following carbon tetrachloride poisoning to produce liver cell necrosis and extensive liver damage.[5]

It is quite clear that an elevated blood iodine may be due to hyperthyroidism and previous administration of iodine-containing compounds, among other things.[7, 8] Chemical laboratories doing blood iodine determinations usually recommend that patients do not receive any iodine-containing compounds for from ten to thirty days prior to the determination of this test.

No information can be found in the literature concerning the quantitative effect on the protein-bound blood iodine of retained Lipiodol in the bronchi and alveoli of the lung as a result of previous bronchograms. Means[9] has noted "the prolonged 'spurious' elevation of protein-bound iodine which follows the administration of such organic iodine-containing compounds as

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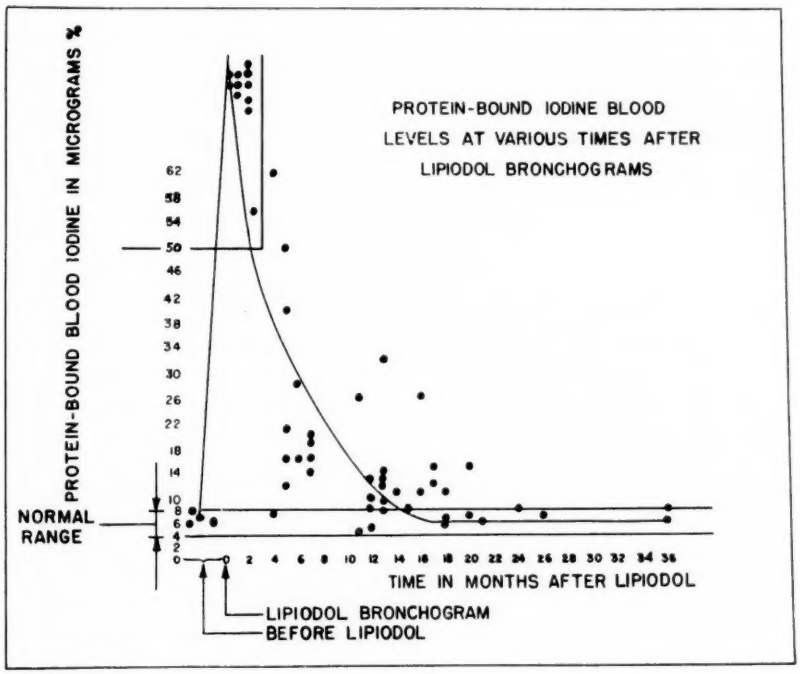
PROTEIN-BOUND BLOOD IODINE IN MICROGRAMS %
PROTEIN-BOUND IODINE BLOOD LEVELS AT VARIOUS TIMES AFTER LIPIODOL BRONCHOGRAMS
NORMAL RANGE
62
58
54
50
46
42
38
34
30
26
22
18
14
10
8
6
4
2
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
TIME IN MONTHS AFTER LIPIODOL
LIPIODOL BRONCHOGRAM
BEFORE LIPIODOL

Fig. 1.

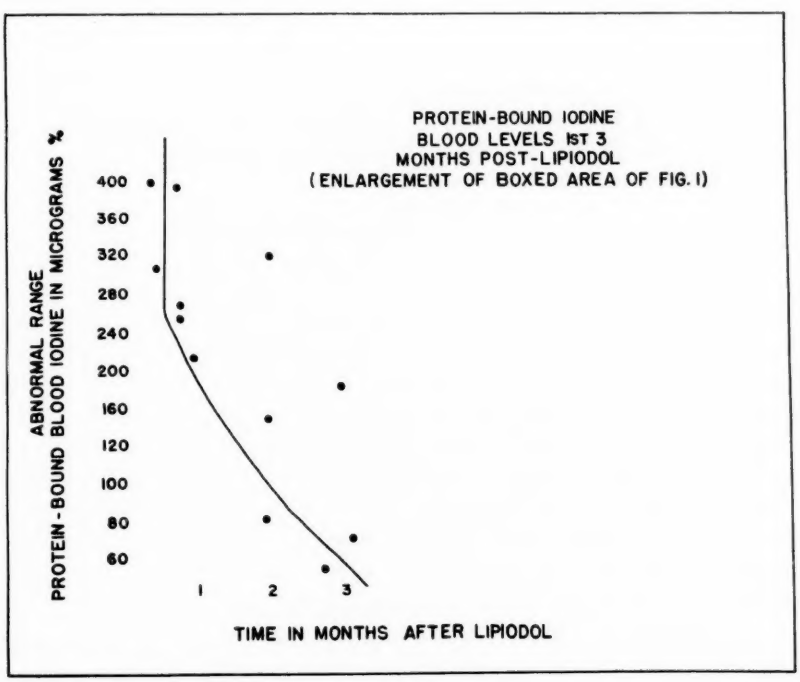
PROTEIN-BOUND IODINE BLOOD LEVELS IST 3 MONTHS POST-LIPIODOL (ENLARGEMENT OF BOXED AREA OF FIG. I)
ABNORMAL RANGE
PROTEIN-BOUND BLOOD IODINE IN MICROGRAMS %
400
360
320
280
240
200
160
120
100
80
60
1
2
3
TIME IN MONTHS AFTER LIPIODOL

Fig. 2.

Iodeikon or Lipiodol used in roentgenography." Salter[10] has stated that "the intrathecal injection of Lipiodol may form a localized reservoir of iodine which will slowly feed small amounts of iodine into the general circulation over the course of many weeks." Communication with several chemical laboratories and with the manufacturers of Lipiodol indicates that the quantitative effect

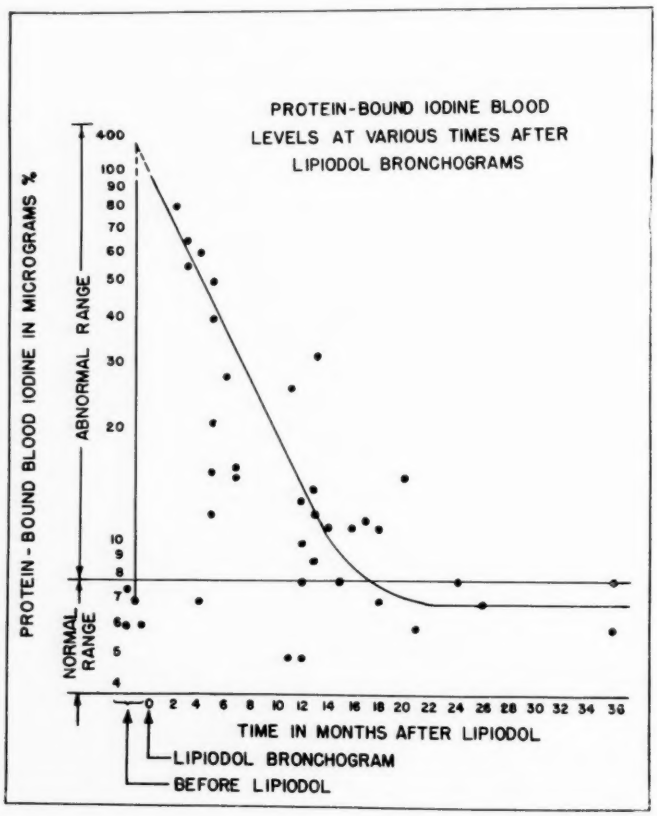

Fig. 3.

of retained Lipiodol is unknown and no studies have been published. The purpose of the present work is to determine the effect of retained Lipiodol upon the blood iodine.

Forty-one determinations of protein-bound blood iodine were secured on thirty patients at varying known intervals following the instillation of Lipiodol* for a bronchogram. Four patients had normal protein-bound blood iodine determinations before Lipiodol instillation (6, 6, 7, and 8 μg per cent). It soon became apparent that retained Lipiodol causes an elevation of the blood iodine† for prolonged periods of time. Values of over 400 μg per 100 ml. of blood were secured one week following the instillation of Lipiodol. The

*Lipiodol 40 per cent containing 540 mg. of iodine by weight per milliliter was used. Varying amounts from 5 to 20 ml. were instilled for visualization of the bronchi.

†"Blood iodine" refers to protein-bound blood iodine as determined by the method of Barker,[11] modified by M. E. Morton using 10 per cent trichloracetic acid to precipitate plasma protein.

protein-bound blood iodine level dropped rather rapidly to average approximately 200 μg per 100 ml. of blood one month after instillation of Lipiodol (still greatly elevated) and then gradually dropped further for some time. It is important to note that the curve drawn of the various values of blood iodine plotted against the time since the last bronchogram did not reach the normal range (4 to 8 μg per 100 ml. blood) until seventeen months after the patient's last bronchogram. (Figs. 1, 2, and 3.)

SUMMARY AND CONCLUSIONS

1. Instillation of Lipiodol for a bronchogram produces extremely high levels of protein-bound blood iodine for prolonged periods (months).

2. It requires approximately seventeen months for the blood iodine level to reach normal in patients who have received bronchograms with Lipiodol. Individual patients may have elevated blood iodine values for three and four years.

3. Blood iodine cannot be used as a diagnostic agent (e.g. in suspected cases of hyperthyroidism) in patients who have had Lipiodol bronchograms within the previous two years, and possibly longer.

Grateful acknowledgment is made to Ellen M. Bahr for the blood iodine determinations.

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THE EFFECT OF THYROID SECRETORY ACTIVITY ON THE DISTRIBUTION OF RADIOIODINE IN PLASMA

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WHEN labelled inorganic iodide is administered by mouth, 90 per cent or more is absorbed in ninety minutes.[1] During this interval, and for several hours thereafter, a portion of the administered dose diffuses into the extracellular fluid. While absorption and diffusion are occurring and during the ensuing twenty-four to forty-eight hours almost all of the iodide ion is competitively removed from the blood and body fluid by the thyroid gland and the kidneys. That which is trapped by the thyroid is soon synthesized into hormone. A variable portion of the latter is stored in the gland while the remainder is secreted into the blood. Once thyroid hormone is released into the blood it apparently undergoes a slow degradation at the rate of about 5 per cent of the existing level per day.[2] Both the rate at which the thyroid gland removes iodine from the blood and the total amount ultimately retained by the gland are characteristically influenced by the state of thyroid activity provided the gland has not been previously saturated with iodine. Each has been measured repeatedly in human subjects by direct and indirect techniques.[3, 4] However, a more positive measurement of thyroid function would necessitate an estimation of the actual rate of hormone secretion. This present study is, therefore, chiefly concerned with the problem of the rate of release of hormone and is based upon serial determinations of radioiodine in the organic fraction of plasma at successive intervals after the administration of a tracer dose of I^{131}.

METHODS

Subjects selected for study included patients with either hyperthyroidism or myxedema, along with a group of euthyroid subjects to serve as controls. In none of the patients was the diagnosis equivocal because of grossly atypical clinical findings or inconsistent laboratory data. No subject had received iodine therapy within six weeks or antithyroid drugs within two weeks before the experiments were started.

The tracer dose of I^{131} was given orally and varied from 0.1 to 0.2 mc. except for occasional patients with hyperthyroidism who received 0.4 millicurie. No normal subjects received more than 0.1 millicurie. The material was either carrier-free or contained not more than 0.5 mg. of NaI. Blood samples were drawn at intervals of two, eight, and twenty-four hours after ingestion of the tracer dose. These were oxalated and a 5 c.c. portion of plasma precipitated with 45 c.c. of Somogyi's zinc sulfate-sodium hydroxide reagent (45 c.c.) as described by Man and associates.[5] After centrifugation, a 20 c.c. aliquot of supernatant was removed and evaporated to dryness in a 2 oz. ointment tin (29 sq. cm. area) by the aid of an infrared lamp. The activity of this fraction was considered to represent that of inorganic iodine.

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TABLE I. PLASMA LEVELS OF I^{131} AS PER CENT OF TWO-HOUR INORGANIC LEVEL

		INDIVIDUAL VALUES													MEAN VALUES	
		HYPERTHYROIDISM														
	PATIENT	L. E.	L. B.*	J. P.	E. N.*	E. J.	E. M.	W. P.	A. P.	P. B.	E. F.	L. P.	E. Fl.*	I. K.*		
Inorganic fraction	2 hr.	100													100	
	8 hr.	14.0	3.4	–	46.0	24.0	4.9	3.6	11.0	17.0	11.0	7.2	42.0	4.3	15.7	
	24 hr.	0	0	3.7	20.0	0.5	2.0	3.9	0	1.3	8.4	3.3	7.0	0.6	3.9	
	Slope 2-8 hr.	-14	-16	–	-9	-13	-16	-16	-15	-14	-15	-16	-10	-16	-14	±0.69†
Organic fraction	2 hr.	15.0	25.0	4.0	1.5	2.5	0.8	12.0	33.0	8.0	–	8.7	4.8	4.0	9.9	
	8 hr.	4.1	1.2	–	0.3	2.9	1.9	5.0	49.0	5.2	5.6	14.0	1.1	7.7	8.2	
	24 hr.	6.2	4.0	18.0	0.0	2.7	5.4	21.0	73.0	8.2	15.0	30.0	2.4	9.2	14.8	
	Slope 8-24 hr.	+0.13	+0.17	–	-0.02	-0.01	+0.22	+1.0	+1.5	+0.19	+0.59	+1.00	+0.08	+0.09	+0.41	±0.14
		NORMAL SUBJECTS														
	PATIENT	A. M.	I. P.	W. F.	M. M.	W. G.	I. L.	G. M.	E. L.	J. B.	A. P.	C. B.				
Inorganic fraction	2 hr.	100													100	
	8 hr.	66	56	47	64	75	69	71	69	31	43	37			57.2	
	24 hr.	19	15	12	28	17	34	12	28	4	8	3			16.3	
	Slope 2-8 hr.	-6	-7	-9	-6	-4	-5	-5	-5	-11	-6	-10			-7	±0.69
Organic fraction	2 hr.	5.1	2.4	1.6	1.2	8.6	23.0	20.0	9.5	3.3	0.6	15.0			8.2	
	8 hr.	3.0	1.4	1.3	1.8	3.4	11.0	–	11.0	1.3	0.3	9.7			4.4	
	24 hr.	1.7	0.2	0.4	0.8	5.1	3.7	1.2	5.2	0.7	0.1	5.1			2.2	
	Slope 8-24 hr.	-0.08	-0.08	-0.06	-0.06	+0.11	-0.46	–	-0.36	-0.04	-0.01	-0.29			-0.13	±0.05
		MYXEDEMA														
	PATIENT	A. H.	L. J.	J. B.	I. R.	L. H.										
Inorganic fraction	2 hr.	100													100	
	8 hr.	68	57	96	60	105									77	
	24 hr.	27	20	76	22	96									48	
	Slope 2-8 hr.	-5.3	-7.2	-0.7	-6.7	0.0									-4.0	±1.5
Organic fraction	2 hr.	6.2	5.8	6.6	3.8	1.0									4.7	
	8 hr.	4.8	3.0	7.2	2.3	1.6									3.8	
	24 hr.	3.5	1.6	8.8	0.9	1.2									3.2	
	Slope 8-24 hr.	-0.08	-0.09	+0.10	-0.09	-0.02									-0.036	±0.036

*Nodular glands (all others are diffuse).
†Standard error of mean.

The protein precipitate which contained organic hormonal iodine was washed three times with 50 c.c. portions of water. Assays of the washings on six different blood samples indicated that any additional washing would not remove more activity than 0.5 per cent of that in the total supernatant fraction from which the precipitate was removed. The washed precipitate was quantitatively transferred to a weighed ointment tin and dried under the heat lamp: It was then distributed evenly over the bottom of the tin with the help of a stirring rod and a small quantity of acetone. The half-density of the precipitate in the containers averaged only 10 mg. per square centimeter.

Counts were made under a Geiger-Müller tube with a 6 mg. per square centimeter mica window, 6.2 cm. in diameter and connected with a scale of 8 counter. An appropriate-sized aliquot of the original material was counted at the same time so that decay calculations were unnecessary. Aliquots of the standard and inorganic fraction were combined with 1 c.c. of a solution recommended by the Oak Ridge National Laboratory to prevent loss of volatile iodine.*

RESULTS

Individual values are recorded in Table I, and composite curves of the inorganic and organic fractions are depicted in Fig. 1. The value of the inorganic fraction at two hours was arbitrarily set at 100 per cent as reference for the remaining determinations. Since total blood or body iodine content was not known, there was no advantage in employing the original dose as a standard for comparison.

The inorganic fraction is seen to decline much more rapidly in hyperthyroid patients than in normals. This is presumed to be due to the more rapid rate of removal by the thyroid gland, provided there is no significant increase in the rate of renal removal. It is of interest that the decrease of plasma iodine with time in the three curves does not give a straight line of constant slope on a semilogarithmic plot. Without more points on the curve it is impossible to come to any exact conclusions, but it may well be that this reflects the sequential operation of several biologic processes, each in itself expressed by a true exponential function.

A more rapid decline is present between the second and eighth hour than between the eighth and twenty-fourth. The initial rapid fall could be due to two factors. (1.) The passage of iodine into the extracellular fluid in addition to the thyroid and the urine. (2.) An exaggerated peak of the blood concentration if the rate of absorption should materially exceed the rate of diffusion into the extracellular fluid. The slope of the three curves between the second and eighth hour when inorganic iodine is being disposed of most rapidly may be seen in the table and in Fig. 1. The difference between hyperthyroid and normal subjects is very real. However, the five patients with myxedema, although showing a flatter average curve, do not differ from the normals to a statistically significant degree.

The behavior of the organic fraction likewise is not the same in hyperthyroid subjects as in normals. Between the eighth and twenty-fourth hour the patients with hyperthyroidism show a distinct rising trend, while the curve of the normal subjects tends to decline. This difference in behavior of the two curves is a real one, as an analysis of the slopes of the two groups

*$NaHSO_3$, 500 mg.; NaI, 250 mg.; NaOH, 750 mg.; water to 1,000 c.c.

will reveal (Table I). The trend of the organic fraction in myxedematous patients is not significantly different from that of the normal group. An observation to be discussed later is the high activity which was encountered in the organic fraction at the two-hour period.

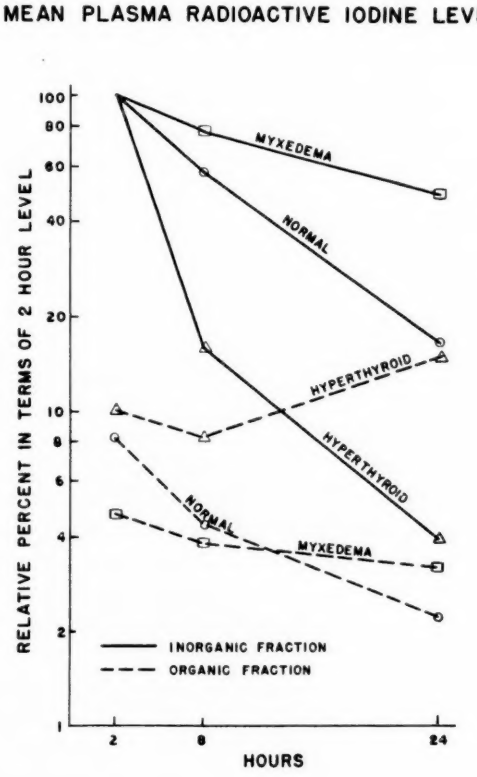

Fig. 1.

In four of the hyperthyroid patients and four normals additional measurements were made after forty-eight hours. The trend of the organic and inorganic curves during this interval was similar to that observed between the eighth and twenty-fourth hour.

DISCUSSION

McConahey, Keating, and Power have recently reported their results of blood fractionation studies in a small series of patients and normal subjects who were given doses of I^{131} ranging from 1 to 100 millicuries.[6] Our own findings are similar in some respects to those reported by this group of workers. The chief point of difference is that the organic fraction in their three normal patients and also in the one hypothyroid patient showed a progressive rise, albeit with a more gradual slope than in the series of hyperthyroid cases. The curves for both normal and hypothyroid patients presented here show a slight progressive fall in activity of this fraction. The reason for

this decline cannot be determined with any degree of precision; however, certain general principles should be noted. There is a slow process of disintegration of thyroid hormone in the body fluids which should proceed at approximately equivalent rates in all subjects. In hyperthyroidism, with relatively little hormone stored in the gland and a relative high uptake of radioiodine, it is to be expected that the material secreted after a tracer dose would be proportionately rich in radioactive iodine. On the other hand, with a normal gland, the comparatively large amount of preformed, nonradioactive hormone would force the gland to deliver to the blood a product relatively poor in the newly acquired radioactive component. Thus it is conceivable that in its normal state of activity the quota of radioactive hormone delivered to the blood would be insufficient to result in a steadily mounting activity within the organic fraction. The rate of change or organic iodine in the blood, therefore, does not offer a direct measure of true secretory activity. That the behavior of the organic fraction is quantitatively influenced by thyroid hyperactivity is nevertheless clearly demonstrated by the difference in slope of the curves as shown here.

The finding of small but unmistakably real quantities of active material in the organic fraction of patients with myxedema in the present study, and in that of McConahey and co-workers, is of interest. Moreover, our own finding of a relatively high value as soon as two hours after ingestion with a decline during the next six hours even in the presence of hyperthyroidism should also be commented upon. An explanation of the presence of active material in myxedematous patients, along with the temporarily high level in all patients, might suggest an extrathyroidal synthesis of an organic iodine complex. Incubation of serum with inorganic iodine under physiologic conditions has not been found to promote such a synthesis.[6] Morton and coworkers, on the other hand, have demonstrated the in vivo production of thyroxine and diiodotyrosine in thyroidectomized rats as early as two hours after the administration of labelled inorganic iodide.[7] It is difficult to believe, however, that the high level of serum activity here encountered at two hours could be achieved so rapidly by extrathyroidal synthesis. Moreover, the initial drop in the curve (between two and eight hours) is too rapid to be attributed to simple thyroxine decay. There is a possibility that a portion of the protein-bound material is not actually a hormonal compound but that it is fixed to protein fully as firmly and resists removal by washing.

The usefulness of a determination of blood radioiodine as a diagnostic test cannot be determined on the basis of the present observations. Significant differences between mean values can easily be demonstrated, but an overlap exists. The steepest slope for the curves of inorganic iodine (two to eight hours) in the normal group was –11 (Table I). However, two hyperthyroid patients (E. N. and E. Fl.) showed slopes which were less steep. Both patients suffered from a relatively chronic type of disease of only moderate severity and each had a rather large nodular gland. In the case of the organic fraction, if one arbitrarily divides hyperthyroid patients from normals on the basis

of a positive or negative slope between eight and twenty-four hours, the overlap consists of one normal patient with a positive slope and two hyperthyroid patients with slightly negative slopes.

Thus the large majority of our cases show definite differences between normal and hyperthyroid blood values. Just how much overlap actually exists can be shown only by a large series. It may well be that this type of determination will prove a useful adjunct to already existing tests for hyperthyroidism.

Freedberg and co-workers have recently reported that protein-bound radioiodine, twenty-four hours after a tracer dose, was consistently higher in hyperthyroid patients than in normal controls.[8] In the present study the rate of change between eight and twenty-four hours correlated with clinical status more consistently than did the final twenty-four hour level.

The behavior of the inorganic fraction in myxedema was not greatly different from normal and the rate of change of the organic component was not distinguishable from that in the normal group. In our own experience the best diagnostic criterion for myxedema when one employs radioiodine techniques is the failure to demonstrate any local uptake of activity by direct measurement over the gland with a counter.

SUMMARY

After a tracer dose of radioiodine the activity of the inorganic fraction of the blood in hyperthyroidism declines more rapidly than normal, while in myxedema the decline tends to be somewhat slower than normal. The activity of the organic fraction is distinctly elevated in all types of cases at the end of two hours, and then tends to decline between the second and eighth hour. This decline is progressive for the ensuing sixteen hours in both normal subjects and patients with myxedema; however, in patients suffering from hyperthyroidism there is characteristically a progressive rise in this fraction between the eighth and twenty-fourth hour.

We are indebted to Ethel Buchwald Chudzick for technical assistance.

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INHIBITION OF THE GROWTH OF STAPHYLOCOCCUS AUREUS BY HUMAN SEMEN

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THE presence of penicillin in blister fluid of human skin following parenteral and topical application was determined by Dostrovsky, Gurevitch, and Rozansky.[1] In another study the concentration of penicillin in human milk was determined by Rozansky and Brzezinsky.[2] In pursuing these studies further we attempted to determine whether penicillin is excreted in the human semen. It was first necessary to find out whether semen in itself has any inhibitory effect on the growth of bacteria. Preliminary examinations revealed inhibition of the growth of *Staphylococcus aureus* by human semen. The following is a detailed report on a study of the inhibition of the growth of *Staph. aureus* by human semen.

MATERIALS AND METHODS

Twenty-eight specimens of semen obtained from twenty-four men between the ages of 27 and 47 years were examined. The semen of four patients was examined twice. Five of the examined specimens were azoospermic, ten were oligospermic (spermatozoa count lower than 60,000,000 per cubic centimeter), and thirteen were normospermic. The patients applied to the clinic for consultation in connection with sterile marriage. Only one patient (Specimen 21) had received 60 mg. testosterone propionate (six injections of 10 mg.) before examination. All the others had received no previous treatment. In most instances the semen was obtained under as sterile conditions as possible by masturbation in the outpatient clinic, but in four instances specimens were brought to the clinic from outside. Each specimen was tested for bacterial contamination before examination. One specimen was discarded because of contamination by *Esch. coli*. In five other ejaculates a few colonies of *Staphylococcus albus* were found.

The inhibitory effect of human semen on bacterial growth was examined by the cup method used in studies on the concentration of antibiotics.[3] The first test strain was *Staph. aureus*, Heatley, in use in antibiotic studies in this laboratory for a period of four years. In the course of later experiments two additional strains of *Staph. aureus* freshly isolated in the laboratory were used: strain 924 isolated from the urine of a patient with cystopyelitis, and strain 598 isolated from the pus in a case of furunculosis. All three strains had the usual properties of pathogenic strains of *Staph. aureus:* coagulase positive, mannite positive, actively hemolytic.

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Examinations were conducted as follows. One cubic centimeter of a 1:1,000 dilution of a twenty-four hour broth culture of the test strain was added to 20 c.c. of nutrient agar (Difco) and plates were poured. The plates were left at room temperature for four hours, after which five cups, 8 mm. in diameter (external), were placed on each plate. Four cups each received 3 drops of the tested ejaculates and the fifth received 3 drops of a penicillin solution containing 0.1 unit per cubic centimeter. The cup containing penicillin served as a control of the area of inhibition of growth of the staphylococci. The results were read after twenty-four hours of incubation at 37° C. In most examinations two plates were prepared for each specimen examined. The specimens were left at room temperature for four hours before examination to permit the ejaculates to liquify and to become homogenous. When sufficient material was available, residues were stored at 8° C. after the first examination and were re-examined twenty-four hours to seventy-two hours later.

RESULTS

The results of the examinations are summarized in Tables I, II, and III, comprising studies in the Heatley strain, strain 924, and strain 598 respectively.

As may be seen in Table I, twenty-three of the twenty-eight specimens studied with the Heatley strain inhibited the growth of the staphylococcus over an area from 10 to 20 mm. in diameter, while five gave negative results. The positive results include examinations repeated after twenty-four and seventy-two hours. In three instances no inhibition was found at the first examination but the same specimens were positive when examined twenty-four and seventy-two hours later. Of the twenty-three positive specimens, fourteen yielded similar areas of inhibition in both plates and nine specimens yielded positive results in one plate (four of these specimens were examined with one plate only). Specimen 17 was examined in three plates, and areas of inhibition 16 mm. in diameter were observed in all of them. In six instances the semen was re-examined following twenty-four hours of storage at 8° C. Five of these specimens were positive and one was negative. Three of the positive specimens were particularly interesting because they were negative on first examination but yielded a distinct area of inhibition twenty-four and seventy-two hours later. Following two weeks of storage at 8° C. Specimen 14 yielded an area of inhibition similar to that of its first examination. Specimen 21 was negative following one week's storage at 8° C.

The inhibitory effects of eighteen specimens of human sperm on *Staph. aureus* strain 924, isolated from urine, are summarized in Table II.

Fourteen of the eighteen specimens were positive, five in two plates and nine in one plate. Four specimens in this series were negative.

The inhibition of *Staph. aureus* strain 598, isolated from a furuncle, by eight specimens of human sperm is summarized in Table III. Of the eight examinations five were positive, each of them in two plates, and three were negative when examined in one plate only.

TABLE I. INHIBITION OF GROWTH OF STAPH. AUREUS, HEATLEY STRAIN, BY HUMAN SEMEN

SPECIMEN	AGE OF PATIENT	QUANTITY OF SEMEN (C.C.)	SPERMATOZOA (MILLIONS/C.C.)	ZONE OF INHIBITION IN MM. AFTER 4 HR.		24 HR.	72 HR.	
				PLATE I	PLATE II	PLATE I	PLATE I	PLATE II
1	27	1	Azoospermia	17	–	–	–	–
2	25	2	80	14	14	–	–	–
3	30	3	2	19	19	–	–	–
4	29	4	70	11	0	–	–	–
5	32	3	120	11	0	–	–	–
6	38	1	85	14	–	–	–	–
7	29	3	40	10.5	0	–	–	–
8	37	4	30	14	0	13	–	–
9	44	3	6	0	0	12	–	–
10	47	2.5	130	0	0	–	–	–
11	39	8	1	0	–	0	0	0
12	33	3	Azoospermia	0	0	15	12	11
13	27	6	70	0	0	15	15	15
14	39	4	40	14	10	–	–	–
15	26	3	20	0	0	–	–	–
16	28	3	70	0	–	–	–	–
17	30	2	Azoospermia	16	16	–	–	–
18	38	3	60	10	10	–	–	–
19 (1, on second examination)	27	1	Azoospermia	0	0	–	–	–
20 (17, on second examination)	30	1.5	Azoospermia	16	15	–	–	–
21	44	2	50	16	15	–	–	–
22	32	3	45	15	12	–	–	–
23	35	3	70	11	0	–	–	–
24	42	4	35	16	15	–	–	–
25	41	3	108	17	16	–	–	–
26	35	3	80	13	–	–	–	–
27 (6, on second examination)	38	2	85	11	9	15	–	–
28 (18, on second examination)	38	5	65	20	15	–	–	–

–, Not examined.
0, No inhibition.

TABLE II. INHIBITION OF GROWTH OF STAPH. AUREUS, STRAIN 924, BY HUMAN SEMEN

SPECIMEN	ZONE OF INHIBITION IN MM. AFTER 4 HR.	
	PLATE I	PLATE II
10	11	–
11	18	–
12	10	–
13	11	–
14	15	10
15	0	–
16	0	–
17	14	14
18	0	–
19	0	0
20	20	15
21	12	12
22	11	0
23	15	–
24	12	0
25	14	13
26	10	–
28	15	0

–, Not examined.
0, No inhibition.

TABLE III. INHIBITION OF GROWTH OF STAPH. AUREUS, STRAIN 598, BY HUMAN SEMEN

SPECIMEN	ZONE OF INHIBITION IN MM. AFTER 4 HR.	
	PLATE I	PLATE II
14	12	11
16	17	17
17	12	12
20	14	11
21	12	12
22	0	–
23	0	–
28	0	–

–, Not examined.
0, No inhibition.

COMMENT

Human semen has been found capable of inhibiting growth of several strains of *Staph. aureus*. Inhibition was observed in 80 per cent of the examinations done with three strains of *Staph. aureus*. The active principle of the semen was not associated with the spermatozoa, since only the supernatant fluid of the semen was used in the experiments. This is supported by the fact that four of the azoospermic specimens showed inhibitory activity. At this stage of the investigation little can be said as to the nature of the active principle. The factor is stable when stored for days at 8° C. Specimen 14 remained active even after fourteen days. The active principle is thermostable. Specimen 18 remained positive after heating for half an hour at 56° C. Specimens 24 and 25 retained their activity after exposure to 75° C. for fifteen minutes.

Three specimens were negative in the first examination but found to be positive in examinations repeated after twenty-four and seventy-two hours. It may be of interest that these three specimens were not kept at room temperature as is usual in these experiments, but were placed mistakenly in the refrigerator when brought from the outpatient clinic. It may be assumed that the homogenization of these specimens, which takes place at room temperature, was interfered with by refrigeration.

Six specimens of sperm which inhibited *Staph. aureus* had no effect on the growth of *Esch. coli*.

SUMMARY

Twenty-eight specimens of human semen were tested for their inhibitory effects on the growth of *Staph. aureus*. Eighty per cent of the specimens inhibited the growth of this organism. Preliminary studies indicate that the active principle is associated with the liquid part of the semen. Some properties of the active principle in the semen are discussed.

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OBSERVATIONS ON THE USE OF A NEW ANALGESIC, NU-2206 (3-HYDROXY-N-METHYLMORPHINAN HYDROBROMIDE)

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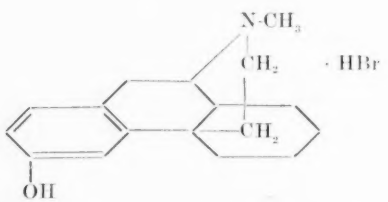
INTRODUCTION

THIS is a preliminary report on a clinical study of the analgesic properties of Nu-2206* (3-hydroxy-N-methylmorphinan-hydrobromide) which includes observations on a series of normal subjects, on a series of patients with postoperative pain, and on a series of patients with intractable pain due to cancer. In instances abstracted in this report it was possible to observe the effect of this drug during and after prolonged use.

CHEMISTRY

Nu-2206 (3-hydroxy-N-methylmorphinan-hydrobromide) has the following structural formula:[1]



Nu-2206 occurs in the form of colorless crystals melting at 193° to 195° C. The substance is rather soluble in water at 20° C. It is easily soluble in water at 100° C. and in alcohol, but it is insoluble in ether. It has analgesic properties similar to those of morphine and some of the other morphine-like compounds developed recently.[2, 3]

ADMINISTRATION TO NORMAL SUBJECTS

The effect on the pain threshold in six normal volunteer student subjects was determined by the Hardy-Wolff-Goodell technique. Doses used were 0.5, 1.0, 2.0, and 3.0 milligrams. Each student received two subcutaneous doses of each of the four graded doses. No subject received more than two doses each week. These doses were compared with doses of 5.0, 10.0, and 15.0 mg. of morphine sulfate given in similar manner and under similar conditions. The results indicated that Nu-2206 was about four times as potent as morphine on a per milligram basis. Peak threshold effects were reached at about the same time as with morphine but there was a somewhat greater duration of effect. The blood pressure changes were no greater than could be accounted for by sedation and resting of the subject.

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*Supplied by Dr. M. J. Schiffrin, Hoffmann-La Roche, Inc., Nutley, N. J.

Doses of 0.5 to 1.0 mg. apparently produced no symptoms in any of the volunteers other than awareness of some vague gastrointestinal distress. Slight sedation may have occurred in some instances. Doses of 2.0 mg. produced some nausea (but no vomiting), dizziness, and sedation. The subjects were aware of gastrointestinal contraction and some inability to concentrate. Doses of 3.0 mg. produced some nausea in practically all subjects; vomiting occurred in two different subjects on one occasion each and dizziness occurred in all subjects. Sedation, inability to concentrate, and awareness of gastrointestinal symptoms were experienced by all subjects. In one subject vomiting occurred five to six hours after the injection. No euphoria was experienced by any of the subjects. In general, 3.0 mg. of Nu-2206 produced fewer and less severe symptoms than an equivalent analgesic dose of morphine. All subjects on 3.0 doses stated they either went to bed right after dinner (five to six hours after injection) or were too sedated to do any studying during the course of the evening. Respiration was not studied, but by casual observation no significant respiratory changes were noticeable.

NU-2206 IN THE TREATMENT OF POSTOPERATIVE PAIN

Nu-2206 was used subcutaneously in a total of fifty-eight postoperative urological and general surgical patients. Thirty-six patients (twenty-nine urological and 7 general surgical) were given 1.5 milligrams. Twelve urological patients were given 4.5 milligrams. Six general surgical patients were given

TABLE I. COMPARISON OF ANALGETIC POTENCIES UPON SUBCUTANEOUS INJECTION

DRUG	DOSE (MG.)	NUMBER OF DOSES	HEIGHT PEAK % RISE THRESHOLD $M \pm \sigma^*$	TIME TO REACH PEAK $M \pm \sigma^*$ (MIN.)	DURATION TO 10% OF PEAK $M \pm \sigma^*$
Morphine SO_4	10	6	21.5 ± 1.9	73.3 ± 8	246 ± 33.2
Morphine SO_4	15	5	27.2 ± 2.5	84.0 ± 7.7	280 ± 7.4
Nu-2206	1	11	10.1 ± 3.0	65.0 ± 9.3	211 ± 13.9
Nu-2206	2	9	18.2 ± 2.1	82.0 ± 5.9	267.0 ± 21.4
Nu-2206	3	9	24.8 ± 2.9	91.1 ± 8.3	301 ± 27.2

*$M \pm \sigma$ is the mean effect $\pm$ its standard deviation.

3.0 milligrams. Four general surgical patients were given 6 milligrams. The majority of the patients to whom 1.5 mg. were given had transurethral resections. These patients usually have very definite discomfort and in some cases severe pain. Ordinarily these patients are given 10 mg. of morphine sulfate immediately after completion of the procedure as the spinal anesthesia is wearing off, and the same dosage is usually repeated in several hours. In these patients Nu-2206 in a dosage of 1.5 mg. was uniformly ineffective. In the patients who had other miscellaneous urologic procedures—three nephrectomies, two suprapubic cystotomies, and one orchidopexy—there was also failure to relieve pain. The one good result in urological patients was in a 9-year-old boy who had a nephrectomy. In the general surgical patients in this dosage range, four obtained relief and three obtained no relief. These seven patients represented a variety of operations. It may be that these surgical procedures did not result

in as much postoperative pain as the average urologic procedure. It appeared that 1.5 mg. of Nu-2206 was an unsatisfactory dose.

In six instances Nu-2206 was used in dosage of 3.0 mg. for treatment of postoperative pain in general surgical patients. It afforded relief in three instances and gave insufficient relief in the remaining three. One of the latter patients subsequently obtained relief of pain with 6 milligrams.

Eleven urological patients who had had transurethral resection were given fifty-one 4.5 mg. doses. However, in this series, Nu-2206 was not given unless the patient had real pain and requested relief. In this dosage range, which was felt to be roughly equivalent to 15 mg. of morphine, Nu-2206 was uniformly effective in relieving pain. Demonstrable effect was noted in most instances thirty minutes after administration of the drug. Analgesic effect was present longer than that observed with morphine and in most instances it was effective for six hours or more. There were no undesirable side effects.

Six milligram dosage was employed in four general surgical patients. In all of the patients to whom 6.0 mg. were given, excellent relief was obtained. The following detailed case is presented as an example of this series.

Patient M. W. (48-9796), a 25-year-old man, was admitted on Aug. 8, 1948, following multiple injuries received in an auto racing accident. Among his injuries were: a perforating wound of the abdominal wall, peritonitis, traumatic rupture of the spleen, severe contusion of the colon, fractures of the transverse processes of the third and fourth lumbar vertebrae, and fracture of left zygoma. Surgical procedures included a splenectomy and colostomy.

TABLE II. PATIENT M. W.

DATE	TIME	DRUG	REMARKS
8/31/48		100 mg. Demerol 5 times per day	Relief
9/ 1/48		100 mg. Demerol 5 times per day	Relief
9/ 2/48		100 mg. Demerol 5 times per day	Relief
9/ 3/48	0300	3 mg. Nu-2206	No relief
	1045	1 mg. Nu-2206	No relief
	1630	3 mg. Nu-2206	No relief
	2000	3 mg. Nu-2206	No relief, *emesis*, excessive perspiration
9/ 4/48	0215	3 mg. Nu-2206	Slept for more than 3 hr.
	1700	3 mg. Nu-2206	Relief from pain for about 3 hr.
	2230	3 mg. Nu-2206	Good relief
9/ 5/48	0230	3 mg. Nu-2206	Slept for 2½ hr.
	0800	3 mg. Nu-2206	Relief
	1520	3 mg. Nu-2206	Relief for about 2 hr.
	2050	3 mg. Nu-2206	Slept more than 2 hr.
9/ 6/48	0030	3 mg. Nu-2206	Slept about 2½ hr.
	0500	3 mg. Nu-2206	Relief
	1545	3 mg. Nu-2206	No relief
	2330	3 mg. Nu-2206	Slept more than 2 hr.
9/ 7/48	0600	3 mg. Nu-2206	Asleep within ½ hr.
9/ 8/48	0345	3 mg. Nu-2206	Asleep within 15 min.

Control of pain was sought by 30 mg. of morphine every four hours during the early stages of hospitalization. This dose was reduced as circumstances allowed, and on August 30, 100 mg. Demerol were substituted for morphine. A tabulation of the drugs given this patient is presented in Table II.

NU-2206 IN THE TREATMENT OF INTRACTABLE PAIN IN CANCER

Five patients with intractable pain due to cancer were observed for varying periods of time up to six months. The following case is presented in detail and is illustrative of some of the effects observed during a prolonged course of administration.

Patient G. M., a 55-year-old white woman, was admitted to the University Hospitals on Oct. 28, 1947, with a diagnosis of carcinoma of the cecum. At operation a carcinoma of the ascending colon with regional lymph node metastasis was found and a right hemicolectomy was performed. She returned to the hospital on Jan. 3, 1949, with a large mass in the right upper quadrant of the abdomen which appeared to be liver involved by metastatic carcinoma. A posteroanterior radiogram of the chest revealed elevation and nodular outline of the diaphragm. The patient had been having very severe pain for two weeks and her physician had been unable to keep her free of pain by intermittent injections of morphine sulfate and Demerol. At the time of her admission she was emaciated and was apparently losing ground rather rapidly, although she was still able to be out of bed for short periods of time. A right splanchnic block was done; this afforded temporary relief of the pain. This was repeated

TABLE III. PATIENT G. M.

DATE	TIME	DRUG	RESULTS
1/ 8/49	1215	6 mg. Nu-2206	Pain relieved in 45 min.
	2015	6 mg. Nu-2206	Pain relieved in 1½ hr.
1/ 9/49	1615	6 mg. Nu-2206	Pain relieved in 45 min.
1/10/49	1700	6 mg. Nu-2206	Pain partially relieved in 30 min.; completely relieved in 60 min. Before: pulse 128, resp. 28 After: pulse 124, resp. 24
1/11/49	1100	6 mg. Nu-2206	No record of result
	2030	6 mg. Nu-2206	Sleeping soundly in 2½ hr. Before: pulse 112, resp. 24 After: pulse 96, resp. 20
1/12/49	0655	6 mg. Nu-2206	Sleeping soundly at 0730
1/13/49	1415	150 mg. Demerol	1515, pain relieved. Almost asleep
1/16/49	0930	150 mg. Demerol	1000, sleeping at intervals
1/17/49	1000	6 mg. Nu-2206	45 min., stated she was not relieved. Later in day stated she spent a comfortable afternoon Before: pulse 120 After: pulse 112
	1930	6 mg. Nu-2206	2010, asleep
1/21/49	1920	6 mg. Nu-2206	2045, no pain Before: pulse 124, resp. 22 After: pulse 120, resp. 20
	2330	6 mg. Nu-2206	0200, asleep
1/22/49	2000	6 mg. Nu-2206	2100, no pain. 2200, asleep
1/23/49	1930	6 mg. Nu-2206	2000, drowsy. 2100, asleep
1/24/49	2000	6 mg. Nu-2206	2100, asleep
1/27/49	1930	6 mg. Nu-2206	2000, dozing
1/28/49	2000	6 mg. Nu-2206	2100, asleep
1/29/49	1930	6 mg. Nu-2206	2000, asleep. Slept all night
1/30/49	1930	6 mg. Nu-2206	2100, asleep
1/31/49	0545	6 mg. Nu-2206	0600, asleep
	2000	6 mg. Nu-2206	Pulse 130, resp. 36. Pulse thready
	2200	6 mg. Nu-2206	Asleep. Expired 2-1-49, 0145

once without satisfactory analgesia. Cordotomy and prefrontal lobotomy were considered but because of the patient's poor general condition and the short life expectancy, it was deemed advisable to try to obtain relief from pain by using analgesic agents rather than to subject the patient to a hazardous operative procedure. As may be seen from Table III, she received a total of twenty-three doses of 6 mg. of Nu-2206 subcutaneously. This was given from January 8 to January 31 when she expired. The degree of relief accomplished by this means was considerable, and particularly gratifying was the long period of analgesia afforded. It was usually possible for her to sleep throughout the night without awakening with pain. This was in marked contradistinction to what had been observed with 10 mg. doses of morphine and 100 mg. doses of Demerol. In two instances during the period the patient was in the hospital, 150 mg. of Demerol were given and the result appeared to be less enduring than with Nu-2206 in a dose of 6 milligrams.

In one urological patient who had an inoperable retroperitoneal tumor with continuous severe pain, Nu-2206 in repeated doses of 4.5 mg. twice daily for a total of 113.5 mg. in twenty-three doses was given throughout his stay in the hospital. He required no other analgesic drug.

In another patient, a 75-year-old white man (Patient E. T.) who had severe pelvic pain following recurrence and local metastasis of carcinoma of the rectum after abdominal perineal resection, Nu-2206 proved to be very effective in producing analgesia. A total of 111 doses, totaling 353 mg., was given over a period of sixteen weeks. Three milligrams proved sufficient when given at bedtime to produce comfort throughout the night so that the patient did not require other analgesic agents or barbiturates for rest. Occasionally during the daytime he took small doses of aspirin (0.6 Gm.) and in a few instances 60 mg. doses of codeine sulfate. During the first five weeks on Nu-2206, he received only eight doses of codeine. After he had received the drug for seven and one-half weeks, complete withdrawal was done for five days. The patient had no symptoms attributable to withdrawal. Examination five days after withdrawal showed no signs indicating withdrawal effect. Due to severe pain the patient was again placed on the drug and the dose was increased to 5 mg. shortly before death. After fourteen weeks he developed symptoms and then signs indicating subtentorial brain metastasis. Three weeks later he expired. No Nu-2206 was given during the last six days of life because of coma.

A summary of our experience with Nu-2206 in the treatment of pain in patients with cancer is presented in Table IV.

Naturally the question of habituation and withdrawal effects is also raised. In addition to the instance already cited (Patient E. T.) in which withdrawal of the Nu-2206 was done after many weeks, ten other patients were studied in this regard. These all received the drug for much shorter periods. None of them demonstrated withdrawal effects. This experience is summarized in Table V.

TABLE IV. NU-2206 IN TREATMENT OF PAIN OF CANCER

PATIENT	SEX	AGE	INDIVIDUAL DOSAGE	NUMBER OF DAYS ADMINISTERED	TOTAL DOSAGE	EFFICACY	SIDE REACTIONS	DIAGNOSIS OR PROCEDURE
C. H.	M	64	6 mg.	4	96 mg.	Maximal	None	Carcinoma of lung (exploratory thoracotomy)
G. M.	F	55	6 mg.	17	126 mg.	Good-maximal	None	Metastatic carcinoma to liver
I. A.	F	78	1.5 mg.	3	4.5 mg.	Maximal	None	Carcinoma of rectum; metastasis and local recurrence
G. W.	F	26	3.0 mg.	3	15.0 mg.	Slight-moderate	None	Mesothelioma of pleura
R. S.	M	58	4.5 mg.	12	113.5 mg.	Maximal	None	Inoperable retroperitoneal tumor
D.	F	32	4.5 mg.	6	88.5 mg.	Good-maximal	Slight dizziness	Carcinoma of cervix
K.	F	40	2.0 mg.	28	20.0 mg.	Slight-moderate	None	Carcinoma of cervix
T.	F	80	2.0 mg.	4	10.0 mg.	Moderate	None	Carcinoma of vulva
C.	F	61	3.0 mg.	4	30.0 mg.	Maximal	None	Carcinoma of cervix
E. T.	M	75	3.0 mg.	35	108 mg.	Maximal	No undesirable ones	Recurrent and metastatic carcinoma of rectum in pelvis

TABLE V. ABSENCE OF WITHDRAWAL EFFECTS OF NU-2206

PATIENT	SEX	AGE	INDIVIDUAL DOSAGE	NUMBER OF DAYS BEFORE DRUG WITHDRAWN	TOTAL DOSAGE	EFFICACY	SIDE REACTIONS	DIAGNOSIS OR PROCEDURE	WITHDRAWAL SYMPTOMS
E. H.	F	34	5 mg.	6	130 mg.	Maximal	Nausea and vomiting, two times	Metastatic epidermoid carcinoma of cervix to 2nd, 3rd, 4th ribs, rt.	None
M. M.	F	82	5 mg.	5	60 mg.	Good	Pulse irregular	Tic douloureux. Nerve section	None
E. S.	M	63	6 mg.	10	84 mg.	Maximal	None	Bronchogenic carcinoma (Rt. pneumonectomy)	None
H. N.	M	53	6 mg.	15	84 mg.	Maximal	None	Metastatic carcinoma in pelvis. Previous abdominal-perineal resection	None
C. B.	M	71	6 mg.	10	78 mg.	Maximal	None	Inoperable carcinoma of lower lobe of left lung	None
E. H.	M	57	5 mg.	2	12 mg.	Maximal	None	Splenectomy for Banti's syndrome	None
E. B.	M	48	5 mg.	7	70 mg.	Maximal	None	Phantom limb pain	None
G. T.	M	52	5.0 to 10.0 mg.	12	590 mg.	Maximal	None	Malignant lymphoma with metastasis to spinal cord	None
R. B.	M	42	3 mg.	4	30 mg.	Maximal	None	Retroperitoneal undifferentiated malignant neoplasm	None
M. B.	F	62	5 mg.	3	40 mg.	Maximal	None	Multiple sclerosis. Cordotomy done	None

SUMMARY

1. Nu-2206 has desirable analgesic properties when administered subcutaneously in doses of 3.0 to 6.0 milligrams.

2. For most adults, 1.5 mg. Nu-2206 is an ineffective dose.

3. The duration of analgesia is more prolonged than with 10 to 15 mg. morphine sulfate.

4. Untoward reactions to Nu-2206 are not severe or frequent.

5. The prolonged effect of Nu-2206 is beneficial in those patients who require protracted relief and in whom frequent injections with shorter-acting analgesics are undesirable.

6. No withdrawal symptoms have been observed in this study.

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THE EFFECT OF SPLEEN PROTECTION ON MORTALITY FOLLOWING X-IRRADIATION

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LEAD protection of the surgically mobilized spleen of CF-1* mice during the delivery of 600 r. total body x-radiation obviates the development of anemia and significantly lessens the severity and duration of the leucopenia and thrombocytopenia that regularly follow total body exposure at this level.[1] On the basis of previous work,[2] a single total body exposure to 550 r. x-radiation produces death of one-half of the mice of this strain in twenty-eight days ($LD_{50}/28$ days).† Survival data on groups of mice exposed to single dosages of total body radiation with or without lead protection of the surgically mobilized spleen are presented in this paper.

MATERIAL AND METHODS

The mice used in this study were all females and were 10 to 12 weeks of age when the experiment was initiated. The mice were kept in the animal farm in a constant temperature room (74° F.) for four to six weeks before use and were maintained on a diet consisting of Derwood‡ and water ad libitum before and after the experimental procedures described below.

Dosimetry.—The x-rays administered in these experiments were generated in a 250 kv. machine operating at 15 milliamperes. A 0.25 mm. copper filter was used. The half-value

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*CF-1, raised by Carworth Farms. Homozygous for the recessive genes aa bb cc.

†Hereafter referred to as LD^{50}.

‡Manufactured by the Derwood Mill, Derwood, Md. Consists of skim milk, ground wheat, yeast, corn oil, salt, iron citrate, shark liver oil, Delsterol.

Table I. Survival of Mice Following Single Total Body X-

Group	Dosage (r)	Spleen Shielded	Total Number of Mice	Number of Survivors*	Per Cent Survival
IV	600	Yes	63	54	85.7
III	600	No	75	30	40
IV	700	Yes	27	26	96.3
III	700	No	11	0	0
IV	900	Yes	60	41	68.3
III	900	No	44	3	6.8
IV	975	Yes	23	10	43.4
III	975	No	12	0	0
IV	1,050	Yes	23	7	30.4
III	1,050	No	11	0	0
IV	1,200	Yes	19	0	0
II	0	Operation only	64	64	100
I	0	No operation	54	52	96

*On Oct. 3, 1949, five or more months after these exposures all mice which had lead protection of the spleen during irradiation still survive and appear grossly normal.

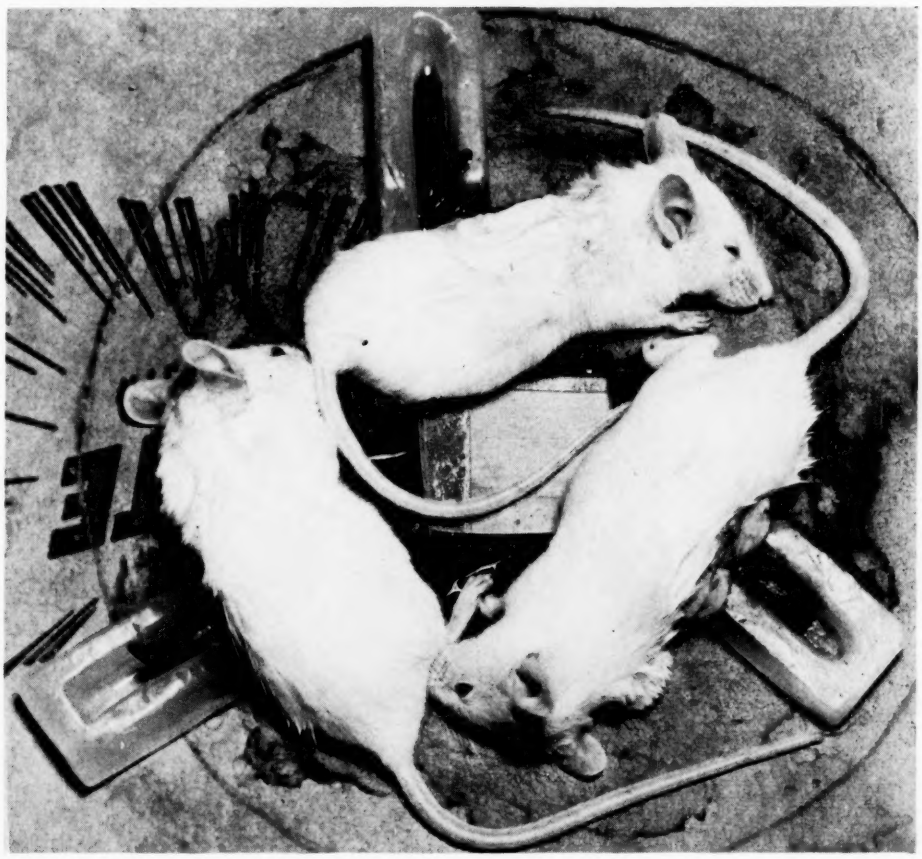


Fig. 1.—Photograph of mice showing surgically mobilized spleens within paraffin boxes. The mice were irradiated in this arrangement.

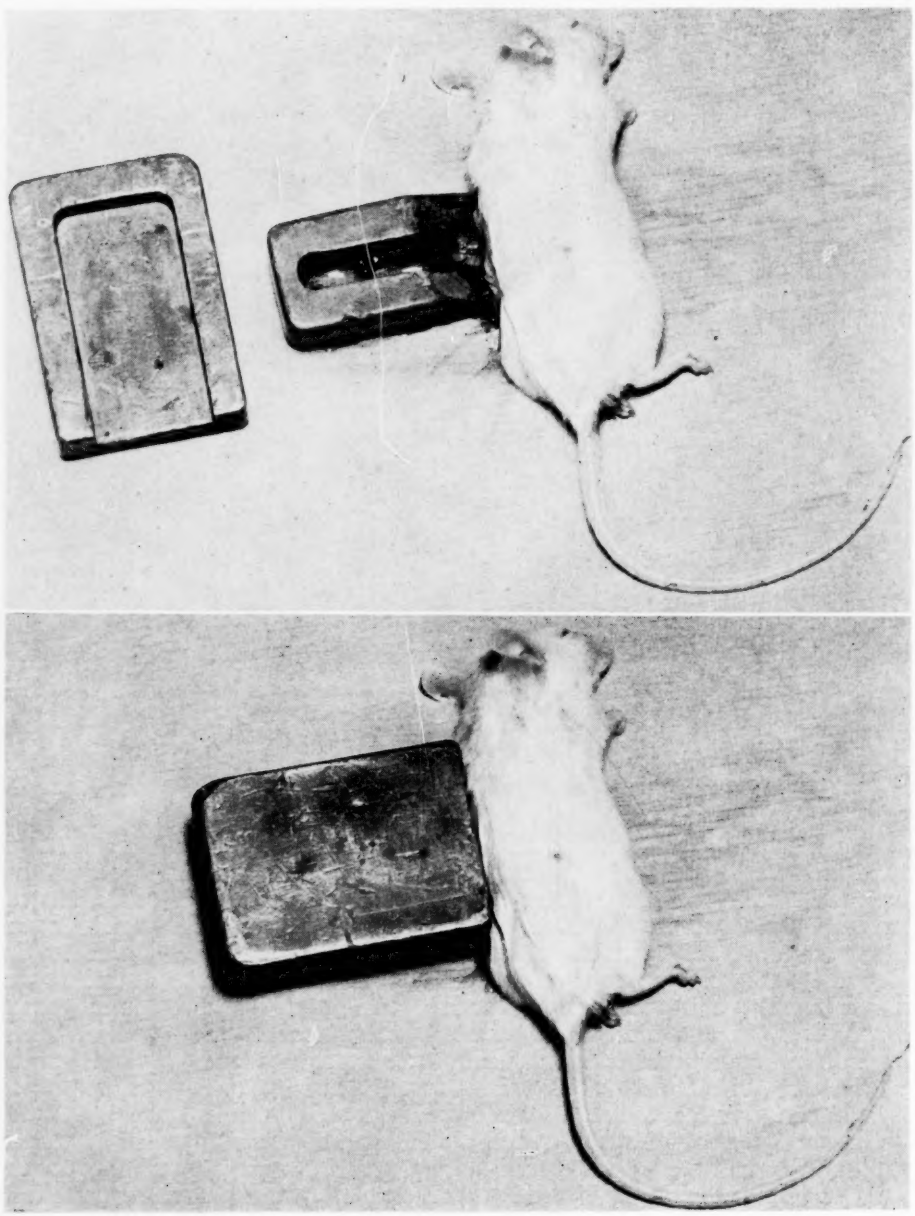
IRRADIATION WITH AND WITHOUT LEAD PROTECTION OF THE SPLEEN

TIME DISTRIBUTION OF DEATHS (DAYS)																									
3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
					2		1	1	2	1	1	1													
		1	3	4	6	4	4	6	4	3	1	1	1	1		4				1				1	
		1																							
			1	2	5	2			1																
	6	3		3			2	1		1					1			1							1
	1	3	6	9	1	12	3	2	2	1	1														
		3	8	1			1																		
1	1	1	3	4	1	1																			
	8	3	5								1														
	4	4	1	2																					
	7	3	1	1	5		1	1																	
			2																						

layer in copper of the filtered beam using 240 kv. was 1.0 millimeter. The exposures were measured with a Victoreen condenser meter equipped with a 250 r. chamber. Measurements were made in air at the position occupied by the center of the animal's body. The dose rate averaged 58.9 r. per minute at 55 centimeters.

Groups of mice were exposed to single doses of 600, 700, 900, 975, 1,050, and 1,200 r. total body x-radiation with or without lead protection of the surgically mobilized spleens.

A.



B.

Fig. 2.—*A*, Photograph of a mouse with mobilized spleen within lead box, and *B*, with lead cover in place.

EXPERIMENTAL PROCEDURE

As is indicated in Table I, four groups of mice were studied. Mice in Group I were untreated controls. The mice in Groups II, III, and IV were anesthetized with Nembutal (0.072 mg. per gram mouse in a 1:10 dilution) given intraperitoneally, the abdomen was shaved, an incision was made in the left upper quadrant, and the spleen was brought out through the incision. The main pedicle was left intact but to facilitate mobilization a small

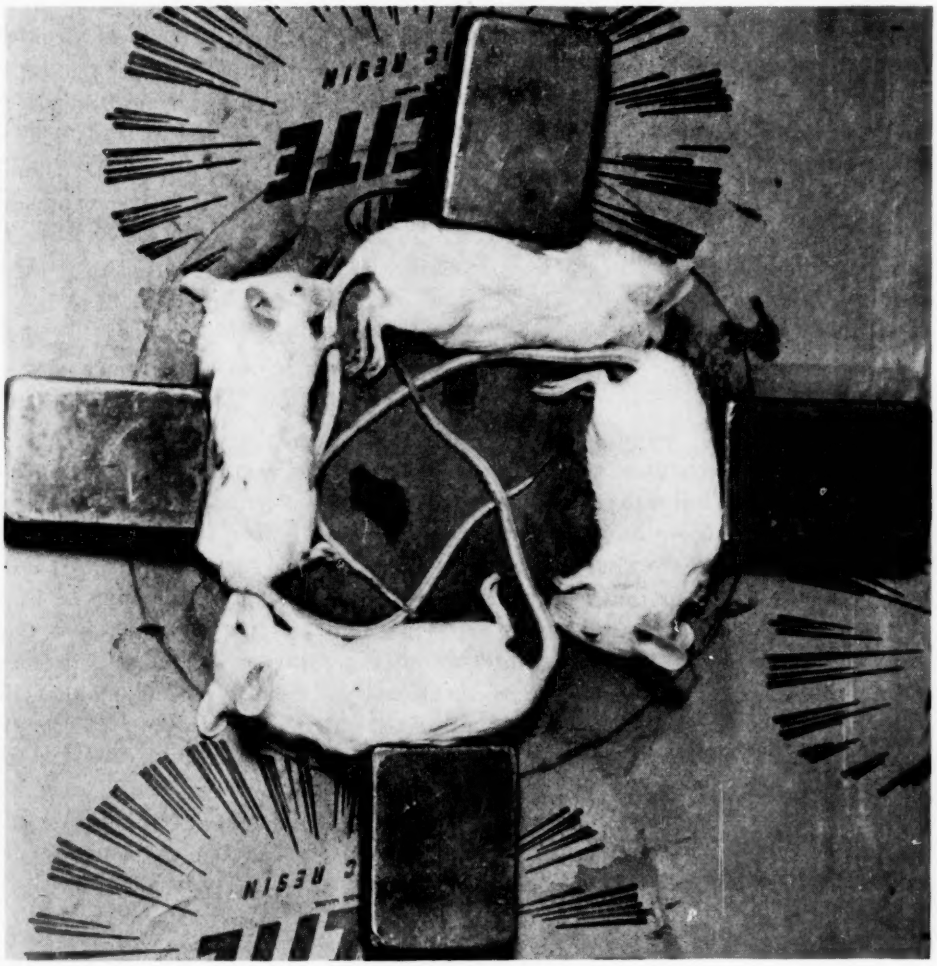


Fig. 3.—Photograph of mice showing surgically mobilized spleens within lead boxes. The mice were irradiated in this arrangement.

vessel at the distal end of the spleen was cut but not tied. Severing this vessel regularly produced an infarct in the tip of the spleen. The infarct varied in size involving up to one-fourth of the distal tip. No significant bleeding was encountered. During irradiation, which required from nine to twenty-two minutes, depending on the dose given, the spleens of Group III mice were placed in paraffin boxes that offered no appreciable shielding (Fig. 1); the spleens of Group IV mice were placed in lead boxes with walls of one-fourth inch thickness (Fig. 2, *A* and *B* and Fig. 3) that afforded essentially complete shielding of the spleen from irradiation. The spleens of Group II were kept in lead or paraffin boxes during the x-irradia-

tion of Groups III and IV. The spleens of all groups thus mobilized were kept moist with physiologic saline during the period in which they were outside the body cavity. After completion of these procedures, the spleens of all groups were returned to the abdominal cavity and the incision was sutured with silk. Complete recovery from the anesthetic required from two to three hours. The mice were returned to the animal farm, and deaths were recorded in twenty-four hour periods through twenty-eight days.

RESULTS

As indicated in Table I, the percentages of survival of animals in Groups I and II (untreated controls and operated controls) were 96 and 100 per cent respectively. With a dosage of 600 r., 40 per cent of the animals in Group III survived the twenty-eight day period. Accordingly, the LD_{50} for animals whose spleens were mobilized but not protected was somewhat less than 600 r. and probably close to the LD_{50} of intact animals (550 r.). On the other hand, in mice of Group IV, which were provided with lead protection of the spleen during irradiation of the balance of the body, a dosage of 975 r. was required to reduce survival to about 50 per cent. In other words, these data show that the LD_{50} for total body x-irradiation exclusive of the lead-protected spleen is nearly twice as great as the LD_{50} for total body x-irradiation inclusive of the spleen.

DISCUSSION

The precise mechanism whereby lead protection of the spleen so significantly increases survival following irradiation is yet to be determined. The capacity of the spleens of these mice to compensate so quickly for the destruction of hematopoietic tissue elsewhere in the body may be the significant factor, but other possible direct or indirect functions of the spleen may be involved. For example, data from a preliminary experiment[3] indicate that rabbits retain the capacity to produce antibodies to intravenously administered foreign red cells (1 c.c. of a 2 per cent suspension of sheep red cells) in a normal manner if either the spleen or appendix is lead protected during the delivery of 800 r. x-radiation to the balance of the body; total body x-irradiation with this dosage but without lead protection of the spleen or appendix more or less completely inhibits the development of demonstrable antibodies to this antigen. The antigen was given two days after irradiation and observations on the development of hemolysin titer followed at intervals of seven days through twenty-eight days.

SUMMARY

Female CF-1 mice of 10 to 12 weeks of age were divided into four groups. Group I mice were untreated controls. Mice in Groups II, III, and IV were anesthetized with Nembutal, an incision was made in the left upper quadrant of the abdomen, and the spleen was mobilized. Mice in subgroups of Groups III and IV were given various single dosages of x-irradiation. During irradiation the mobilized spleens of Group IV mice were protected from radiation by lead shielding, whereas no shielding was provided for the spleens of mice in Group III. Group II mice served as "operated" controls. The dosages administered were 600, 700, 900, 975, 1,050, and 1,250 r. Irradiation time ranged between

nine and twenty-two minutes. After irradiation the spleen was returned to the abdominal cavity and the incision was sutured. The survival of the mice in these various groups was observed over a twenty-eight day period. Without lead protection of the spleen, the LD_{50} for irradiated mice in the twenty-eight day period of observation was less than 600 r., whereas the LD_{50} for mice with lead protection of the spleen approximated 975 r.

CONCLUSIONS

The LD_{50} for mice exposed to total body x-radiation exclusive of the surgically mobilized lead-protected spleen is nearly twice as great as the LD_{50} for mice exposed to total body x-radiation inclusive of the spleen.

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OBSERVATIONS ON MECHANISMS OF EDEMA FORMATION IN THE LUNGS

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INTRODUCTION AND REVIEW OF LITERATURE

KNOWLEDGE of the pathologic physiology of pulmonary edema has been considered fundamental to the understanding and management of clinical heart failure, and the condition has received extensive study. From a great body of experimental and clinical observation, two theoretical conceptions of the genesis of pulmonary edema from heart failure have emerged.[1, 2] First, many observers have stated that pulmonary edema depends on failure of the left ventricle (the right remaining essentially competent), leading to congestion and edema of the lungs. The second concept has held that pulmonary edema occurs when the pulmonary vascular bed becomes widely dilated from noxious or aberrant reflexes permitting inundation of the lung tissue. This concept has further held that pulmonary edema is not caused primarily by failure of the left ventricle. The importance of the problem of pulmonary edema has seemed to warrant a re-exploration of the condition from the experimental standpoint.

In 1878 Welch[3] stated, in his now famous paper, that severe strain imposed upon the left ventricle by gradual occlusion of the aorta resulted in lung congestion and edema. His experiments indicated that ligation of the aorta distal to the large vessels arising from the arch did not restrict left ventricular output sufficiently to strain the chamber. However, if the vessels to the head and upper extremities were tied off one by one after occlusion of the aorta distal to these vessels, the vascular bed was finally reduced so as to overwhelm the left ventricle; marked pulmonary congestion and transudation followed. Subsequently, a number of German workers were able to offer general confirmation of Welch's observations. Sahli[4] found that obstruction of pulmonary venous drainage by constriction of the left auricle was attended by an outpouring of fluid into the lungs. Sahli was unable to reproduce Welch's results by ligation of the aorta, but it is not clear from his paper where aortic constriction was applied. Other observers[5, 6, 7, 14] were able to show that experimental maneuvers capable of overloading the pulmonary venous circuit could precipitate pulmonary edema. Modrakowski[8] demonstrated that isolated lungs could withstand high arterial perfusion pressures without apparent harm, as long as venous outflow was unimpaired. On the other hand, when the lobes were congested by the prevention of venous outflow, only a moderate elevation of perfusion pressure was necessary to provoke edema.

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Similar experiments were carried out on denervated heart-lung preparations.[9, 10, 11] The data from these experiments indicated that as left ventricular output was diminished (e.g., from cardiac damage by asphyxia, "toxic factors," or acute strain), venous engorgement of the lungs occurred, ultimately causing severe edema. The work of Barry[12] was of particular interest. He observed in heart-lung preparations that, if the viscosity of the blood was decreased by the addition of saline, edema of the lungs could be induced. This effect was apparent when the specific gravity of the blood was reduced from 1.053 (normal) to 1.045 or 1.050. However, Barry also observed in these preparations that excessive venous inflow, together with high peripheral arterial resistance, overloaded the left ventricle so that congestion and alveolar edema ensued. Barry's observations were of significance in their demonstration that the Starling principles[13] of vascular pressures and tissue fluid balance may apply to the lung as well as to structures supplied by the peripheral vascular system.

On the basis of such experiments, the concept of pulmonary engorgement and transudation from predominant left ventricular failure has been widely accepted, and much of the rationale of the management of clinical heart failure has rested upon this premise.

Many observers, however, have accepted the alternative concept that pulmonary edema may result from intense reflex vasodilatation of the vascular bed of the lungs or from increased capillary permeability. They have argued that failure of the heart may not be primarily concerned in initiating pulmonary edema. Lambert and Gremels[15] insisted that the formation of edema in heart-lung preparations was accompanied by only a slight rise of pulmonary venous and arterial tensions. These pressures became significantly elevated after edema was established. They thought the capillary leakage was caused by the "toxicity" of the shed blood used in the preparations. A number of others[11, 16, 17, 18] have shared the view that toxic factors occurring in the blood may injure the pulmonary capillaries and facilitate transudation.

Cataldi[29] used a technique similar to that of Coelho and Rocheta,[14] and he noted the precipitation of pulmonary edema when portions of the right ventricle were destroyed by solutions of silver nitrate or absolute alcohol introduced into the ventricular wall. He contended that failure of the left ventricle was not a necessary prerequisite to the onset of pulmonary edema, for damage to the right ventricle might be equally deleterious to the lungs.

The contention that pulmonary vascular reflexes may lead to lung edema formation has rested largely on observations entailing marked disturbances of the sympathetic nervous system.[19, 22-27] Luisada[19] produced paroxysms of edema in rabbits by the use of adrenaline. The paroxysms were often prevented by the use of sedatives (i.e. morphine or the barbiturates), or by transsection of the spinal cord at the cervical level. He attributed these effects of adrenaline to stimulation of the central nervous system including centers controlling pulmonary vascular reflexes and capillary permeability. In contrast, evidence has been evolved to show that adrenaline may seriously affect the heart[20] and provoke myocardial failure.[21] Luisada and Sarnoff[22] declared that experimental

procedures producing cerebral anemia may alter the distribution of blood within the lungs by vasomotor mechanisms, so that alveolar transudation occurs. A similar explanation was suggested by Jarisch and co-workers[23] for the edema resulting from the intracisternal injection of veratrine in rabbits. Farber[24] reported that edema of the lungs frequently occurred in guinea pigs following bilateral cervical vagotomy causing the loss of vasomotor control of the lungs. However, Sussman and co-workers[28] could find no such effect of vagotomy in guinea pigs when respiration was optimally maintained.

On the basis of clinical observation, a number of workers have suggested that pulmonary edema may be touched off by failure of autonomic control of the pulmonary vascular bed. Salmon[30] implicated stimulation of the carotid sinus as a means of inducing transudation. Intracranial hemorrhage[31] and injuries of the spinal cord[32] and of the medulla oblongata[33] have likewise been reported to cause intense pulmonary edema.

In most of the clinical and experimental observations of "neurogenic" pulmonary edema, measurements of cardiac performance were not reported. If the function of the heart were seriously curtailed through aberrant autonomic stimulation from various causes, one might expect lung congestion and its consequences from a critical decrease of left ventricular output. In fact, Campbell, Haddy, and Visscher[34] have shown recently that elevation of intracranial tension may be accompanied by marked bradycardia, diminution of cardiac output, and elevation of pulmonary venous and arterial pressures, with the occurrence of pulmonary edema. These responses were modified by severance of the vagus nerves.

The conflicting views of the genesis of pulmonary edema, as indicated in this review, have not been reconciled. Luisada has stated that "the generally unsatisfactory basis for the backward failure theory of pulmonary edema strongly favors its abandonment."[19] On the other hand, there is a paucity of evidence to support the "neurogenic theory" as the sole cause of edema of the lungs. Furthermore, other evidence has indicated that the latter concept must be viewed with skepticism until reflex effects upon the heart and its function have been investigated more thoroughly.

It seems clear however that any attempt to re-explore the cause of pulmonary edema requires an understanding of the means by which abnormal fluid exchange may occur in the lungs. Although the concise precepts of fluid balance described by Starling[13] are widely accepted as applying to the lung as well as to other tissues, there has been little controlled observation of the Starling principles on the lungs. Experiments were therefore designed to test the effects of increased capillary hydrostatic pressure and of diminished osmotic tension (the Starling principles) on pulmonary circulatory function. Experiments dealing with this aspect of the problem will be reported in this paper.

METHOD

Experiments were performed on dogs under Nembutal anesthesia. The right thoracic duct was isolated, and the rate of lymph flow was measured by the method previously described by us.[35] Under positive pressure respiration, the thorax was opened and the

pericardium incised. The animal was then heparinized in the usual manner, after careful hemostasis had been secured. Systemic and pulmonary arterial pressures were recorded from cannulae placed in the left subclavian artery and right upper pulmonary lobar artery; these were connected to simple mercury manometers. A water manometer was attached to a cannula tied into the left atrial appendage. Ordinary heart-lung preparations were then set up.

In order to study the effects of decreased osmotic pressure, the protein content of the blood was reduced by partial replacement of the plasma with Locke's solution (six experiments). Dilution was accomplished by centrifuging a quantity of blood approximately equal to that in the circuit; the plasma was removed to the level of the packed cells by suction, and the original plasma volume was then restored by the addition of warmed Locke's mixture. At any given time, the normal blood in the venous reservoir could be drained away and replaced by the red cell suspension. The rates of lymph flow, the various pressures, and the appearance of the lungs were repeatedly observed. Sections of the lungs for histologic examination were taken during and at the termination of the experiments. Plasma protein levels were determined by the methods of Campbell and Hanna[36, a] and of Howe.[36, b]

The effect of increased pressure in the pulmonary capillaries on edema formation was studied in six preparations. Pulmonary engorgement was produced by "overloading" the left ventricle. Elevation of venous inflow (and therefore cardiac output) or increase of the peripheral resistance, singly or together, produced left ventricular dilatation and left atrial engorgement. The effects of this treatment on pulmonary arterial and venous pressures, and on blood volume, were noted. Lymph flow was frequently determined, and sections for microscopic study of the lungs were taken.

All specimens of lung tissue were fixed in formol-saline solution (Trowell[37] so as to facilitate preservation of edema fluid and were stored in 80 per cent alcohol.

RESULTS

Effects of Diminution of Plasma Proteins on the Formation of Pulmonary Edema.—During the control periods, the heart-lung preparations functioned under loads that appeared to place no strain upon the myocardium. Usually the peripheral resistance was adjusted to produce a mean aortic pressure of 90 to 110 mm. Hg; the cardiaic inflow was about 400 c.c. per minute. Under these conditions, mean pulmonary arterial pressure varied from 10 to 20 mm. Hg and left intra-atrial tension ranged from 3 to 6 cm. of water. Thereafter the suspension of cells in Locke's fluid was placed in the venous reservoir after removal of as much of the normal blood from the circuit as possible without introduction of air into the venous tubing. Usually 200 to 300 c.c. of the cell suspension were used. In most experiments, it was estimated that approximately one-half of the circulating blood volume was replaced when these quantities of cell suspension were used.

The effects of the addition of diluted blood were first manifested in the flow of lymph from the lungs. The flow increased notably within one to ten minutes, and thereafter was progressively accelerated. The level of blood in the venous reservoir decreased, indicating a loss of circulating blood volume. However, the size of the heart was not increased, and pressures in the pulmonary arteries and left atrium were not materially altered. In four experiments, pulmonary arterial pressure was not affected at all; in one instance a decrease of 4 mm. Hg tension was noted, while in two examples there were transient elevations of the pulmonary blood pressure of 5 mm. Hg. Pulmonary venous pressures were unaltered in two instances, and rose from 2 to 6 cm. of water in four

preparations. Therefore, the continued normal size of the heart and the general level of performance indicated that cardiac function was not impaired (Fig. 1 and Table I).

After the cell-saline suspension was circulating in the preparations, and during the period of fluid loss into the lungs, there was a gradual return of plasma protein concentration to normal values. In some instances the plasma

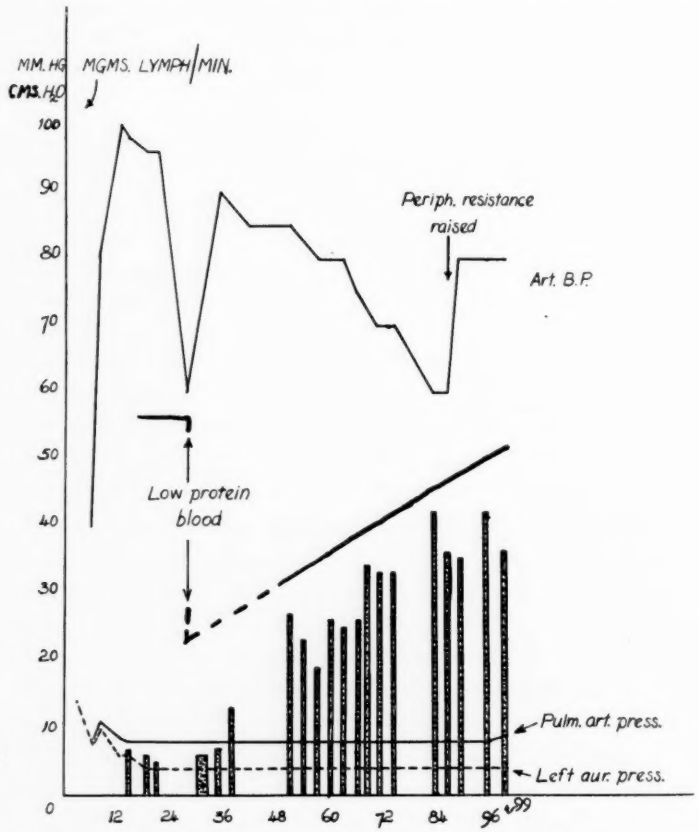

Fig. 1.—The effect of hypoproteinemia upon fluid exchange in the lungs. At the time indicated, low protein blood was placed in the heart-lung system. Protein concentration of the blood is indicated by curve ——— - - - ———. Columns represent lymph flow. Abscissa is time in minutes. See Table I, Experiment 48.

proteins continued to rise to concentrations greater than those in the normal, undiluted blood. (See Table I.) However, with the rise of plasma protein concentrations to control (or above control) values, a corresponding diminution of lymph flow and reduction of pulmonary edema was *not* observed. This phenomenon is well illustrated in Fig. 1. Note that the curve indicating plasma protein concentration of the cell suspension showed a progressive increase, but that lymph flow also showed a continuous rise. The persistence of accelerated lymph flow after the return of plasma proteins to normal levels probably represented the drainage of the previously accumulated fluid from the lungs. However, the mechanism by which the plasma proteins subsequently rose to levels

TABLE I. EFFECT OF DIMINUTION OF PLASMA PROTEINS

EXPERIMENT	CHANGE IN PULMONARY ARTERIAL PRESSURE (MM./HG)	CHANGE IN LEFT AURICULAR PRESSURE (CM./H_2O)	LYMPH FLOW CONTROL (MG./MIN.)	LYMPH FLOW AFTER DILUTION (MG./MIN.)	PLASMA PROTEIN CONTROL (GM. %)	PLASMA PROTEIN DILUTED (GM. %)	PLASMA PROTEIN FINAL (GM. %)	PULMONARY EDEMA
44	+5 to +2	+5 to +3	40	180		3.1	7.0	Present
48	0	0	5	42	5.5	3.1	5.8	Present
49	+5 to 0	+2	6	16	5.2	2.3		Present
51	0	+4	16	78	6.5	2.1	8.5	
53	0	+6			5.5		9.8	Present
54	0	0	98 Av.	150 Av.	7.4		8.2	Present

above control values is not clear. Possibly the interstitial fluid produced by the initial hypoproteinemia obstructed aeration of the lung tissue, causing anoxic damage to the pulmonary capillaries and abetting transudation of protein-poor fluid. Another explanation may be that plasma protein lost into the extravascular space during initial transudation increased tissue fluid osmotic tension to such a degree that transudation went on despite the increase of blood protein level, producing greater hemoconcentration.

Influence of Cardiac Insufficiency and Pulmonary Congestion on the Formation of Edema of the Lungs.—These observations were carried out on six heart-lung preparations. For control purposes, the venous inflow and peripheral resistance were adjusted so as to impose no undue strain upon the hearts, as in the preceding experiments. In four experiments the cardiac load was augmented by increasing the aortic pressure from 90 to 110 mm. Hg to 160 to 180 mm. Hg. In two instances, overburdening of the heart was accomplished by sharp elevations of venous inflow to 600 to 700 c.c. per minute (control, 200 to 300 c.c. per minute). With both means of taxation of the heart, left ventricular dilatation became apparent. The pulmonary venous and arterial pressures then rose and congestion promptly ensued. Congestion of the lungs was manifested by turgescence, followed quickly by duskiness of the tissue. Myriads of fine crepitant râles could be discerned by direct auscultation. The fluid volume in the venous reservoir diminished rapidly, and the passage of lymph from the right thoracic duct progressively increased beginning three to twelve minutes after the onset of congestion. As the process continued, the pulmonary lymph became pinkish in hue, and finally dark red. Microscopic examination of the red lymphatic fluid showed that it contained up to 600,000 red blood cells per cubic millimeter. Sections of the lung tissue taken at the time of accelerated lymph flow exhibited intense congestion and edema. In some instances pulmonary transudation was so marked that foam extended into the main bronchi and trachea. In all of these experiments, pulmonary engorgement and edema was permitted to become extreme; no attempt was made to reverse the process by reducing cardiac load.

The difference in appearance between these lungs and those of the hypoproteinemic experiments was striking. When the hypoproteinemic lungs became edematous, they were pale and flaccid, the acceleration of lymph flow was not so rapid, and the general tempo of the reaction was rather gradual. In contrast, the congested lungs became red and turgid, the acceleration of lymph flow was rapid, and the lymph became hemorrhagic. Edema fluid was frequently profuse.

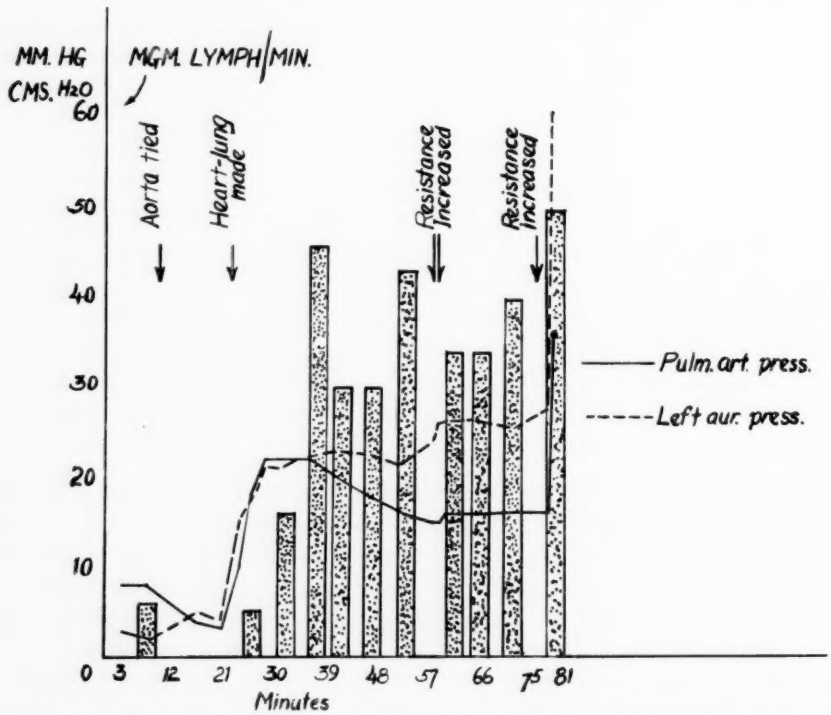

Fig. 2.—The effects of pulmonary congestion upon fluid exchange in the lungs. When the heart-lung was established in this experiment, peripheral resistance was excessive and pulmonary congestion immediately appeared, as indicated by venous and arterial pressure curves. Congestion was further increased at arrows. Columns represent lymph flow.

DISCUSSION

These experimental data suggest that the well-known Starling principles of vascular and tissue fluid balance are operative in the lung structures. The mechanism of pulmonary edema induced by low plasma protein—low blood osmotic tension—is clear and requires no particular comment. However, the production of pulmonary edema from congestion is more important from the standpoint of cardiac insufficiency.

The experiments show that in the heart-lung preparation, left ventricular load may be greatly augmented by elevation of aortic pressure or by increasing the stroke and minute output or by both. Thus the left ventricle can be overloaded so that venous inflow (and right ventricular output) may exceed the greatest possible left ventricular output. With failure of the left ventricle to accept all of the blood flowing to it through the lungs, pulmonary venous

outflow is impaired and blood is impounded in the lungs by the continuing competence of the right ventricle. Examination of Fig. 2 and Table II shows that these events cause pulmonary venous and arterial pressures to rise to high levels. Since the normal pulmonary capillary pressure is estimated at mean values of 10 to 15 mm. Hg in open-chest preparations[38] and the normal blood osmotic pressure at 20 mm. Hg (judged by normal plasma protein levels in these and other[39] experiments), it is apparent that capillary pressure may exceed that of the osmotic tension when the lungs are congested. Rapid filtration of fluid from the capillaries then occurs.

TABLE II. EFFECTS OF PULMONARY CONGESTION

EXPERIMENT	RISE IN PULMONARY ARTERIAL PRESSURE (MM./HG)	RISE IN LEFT AURICULAR PRESSURE (CM./H_2O)	LYMPH FLOW CONTROL (MG./.MIN.)	LYMPH FLOW AFTER CONGESTION (MG./.MIN.)	PULMONARY EDEMA	CARDIAC OUTPUT (C.C./MIN.)
35		59	65	145	Present	
38	36	57	6	63	Present	
39	48	74	40	185	Present	
40	50	84			Present	
41	34	50			Present	300 to 680
43	5	14			Present	160 to 810

A contrasting explanation for experimental pulmonary edema was offered by Luisada and Sarnoff.[22] They reported experiments in which saline infusions, given in large quantity, produced pulmonary edema. They noted that when the solutions were injected cephalad into the carotid arteries under high pressure, edema of the lungs frequently occurred. On the other hand, when comparable amounts of fluid were injected into the systemic veins under lower pressure, edema was somewhat less frequent. They suggested that forcible irrigation of the cerebral circulation by salt solutions resulted in cerebral anemia and provoked pulmonary transudation by reflexogenic influences on the vascular bed of the lungs. Their results were compatible with sudden and serious cardiac overload. They paid scant attention to the possibility of altered circulatory dynamics, and the influence of massive saline infusions on the plasma proteins was not considered.

Other workers[40, 41] have also reported that massive increases in the circulating fluid volume may introduce a load against which the heart cannot cope, with consequent pulmonary congestion and edema. This factor was particularly striking in the studies of Gibbon and Gibbon.[27] In cats, they removed the right middle and both lower lung lobes so as to raise the pulmonary hydrostatic pressure by restriction of the circulatory capacity of the lungs. They found that intravenous saline infusions were especially likely to provoke pulmonary edema and rapid death in these preparations. Although these workers attributed the edema mainly to increased capillary permeability rather than to any dynamic effects, their results suggest that edema occurred from elevation of the pulmonary hydrostatic pressure and possibly from diminution of the plasma proteins.

The correlation of increased lymph flow and the onset and progression of pulmonary edema was strikingly evident in these experiments. We noted that in every instance where a significant elevation of lymph flow occurred, gross or microscopic pulmonary edema could be found.

SUMMARY

Experiments are presented which show that pulmonary edema may be induced in the heart-lung preparation by (1) the lowering of the plasma proteins from replacement of blood plasma with Locke's solution and (2) by elevation of pulmonary vascular (hydrostatic pressures following imposition of left ventricular overload. It was demonstrated that abnormal fluid exchange in the lung tissue may result from disturbances of hydrostatic and osmotic pressure relationships as described by Starling.[13] Although the results of these experiments, involving the heart-lung preparation, do not necessarily explain the cause of acute pulmonary edema in intact subjects, they do demonstrate that Starling's principles apply to the pulmonary as well as to the systemic circulation. The Starling principles, therefore, must be considered in all future studies relating to the pathogenesis of pulmonary edema.

The onset and progression of pulmonary edema were always attended by an increase in the flow of lymph from the right thoracic duct. The measurement of an increased pulmonary lymph flow has been found to be a reliable indicator of the presence of pulmonary edema.

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LABORATORY METHODS

POLYVINYL ALCOHOL-FIXATIVE AS A PRESERVATIVE AND ADHESIVE FOR PROTOZOA IN DYSENTERIC STOOLS AND OTHER LIQUID MATERIALS

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TROPHOZOITES of intestinal amoebae deteriorate rapidly, and consequently it is frequently difficult for a physician to get laboratory confirmation of suspected cases of amoebic dysentery or amoebiasis. Submitting the stool to a distant laboratory for diagnosis has been unsatisfactory since the etiological agent, even if originally present, is usually unrecognizable by the time the specimen is examined. A negative report under these circumstances is, of course, meaningless.

In an attempt to solve this problem, Goldman[1, 2] devised a technique for preserving fecal smears with a film of polyvinyl alcohol (PVA)-fixative. Smears so prepared could be shipped for long distances or stored in a dried condition for months without undergoing any deterioration. In the laboratory, the PVA films were removed and the smears were stained by standard hematoxylin procedures.

During 1948, this method was used by this laboratory in cooperation with local health officials and physicians in Georgia, Alabama, Mississippi, Kansas, North Dakota, Minnesota, and California. Out of thirty specimens submitted during several months, a diagnosis of *Endamoeba histolytica* was made in thirteen instances. The rest showed either no organisms or trophozoites and cysts of various other intestinal protozoa (*Endamoeba coli*, eleven; *Endolimax nana*, fifteen; *Dientamoeba fragilis*, one; and *Giardia lamblia*, one).

The purpose of the present paper is to report simplified and improved methods for preparing specimens and for handling them in the laboratory, and to point out possible applications of the technique to the study of organisms other than intestinal protozoa.

MATERIALS AND METHODS

Polyvinyl Alcohol.—Polyvinyl alcohol is a synthetic water-soluble polymer of vinyl alcohol that is available in the form of a white, odorless powder or granular material. Its aqueous solutions are stable over long periods of time. Viscosity of the solutions can be regulated

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by concentration of PVA and by the degree of polymerization of the alcohol.* Films formed by drying aqueous solutions are tough, thin, and transparent. They generally adhere firmly to clean glass surfaces and are resistant to alcohol, ether, acetone, xylol, oils, and even short exposures to water.

PVA has been used as an embedding medium for tissues,[3] as a mounting medium for insects and fungi,[4, 5, 6] and as a means of reducing motility of paramecia and other small organisms.[7]

PVA-Fixative.—The fixative was prepared as described by Goldman.[2] Five grams of powdered PVA were added to a mixture at room temperature containing 1.5 ml. of glycerol, 5 ml. of glacial acetic acid, and 93.5 ml. of Schaudinn's solution (2 parts of saturated aqueous mercuric chloride to 1 part of 95 per cent ethyl alcohol). Heating to approximately 75° C. while stirring facilitated the preparation of a water-clear, nonlumpy solution. This was used as soon as it had cooled to about 50° C. or less and it remained satisfactory for several months.

Preservation of Specimens With PVA-Fixative.—

A. On Microscope Slides: With the aid of an applicator stick, a drop of specimen was mixed on a microscope slide with 3 drops of fixative and smeared over approximately one-third of the glass surface. To insure thorough drying, smears were usually placed in an incubator at 37° C. overnight.

B. In Vials: A quantity of specimen was thoroughly mixed in a vial containing three or more parts of fixative. Smears for staining were prepared immediately or months later by spreading a drop or two of the mixture on a microscope slide and allowing it to dry thoroughly. Care was taken not to have the smears too thick.

Staining of Organisms in PVA Films.—In the original PVA-fixative technique, fecal smears were covered but not mixed with fixative. In the laboratory the films were removed before staining the smears. The present methods of preservation were made practical by the observation that PVA films are permeable to all commonly employed staining reagents. This makes it possible to stain dried PVA smears in the same manner as smears fixed by conventional methods with Schaudinn's fixative.

Inasmuch as critically stained organisms were desired, the long Heidenhain iron-hematoxylin procedure usually was employed. Diagnostically satisfactory preparations were obtained by more rapid staining procedures such as the ones proposed by Tompkins and Miller[8] and Goldman.[9] Delafield's hematoxylin was used in some instances, as in staining Paramecium. In all of the staining procedures, dried films were first placed in 70 per cent alcohol containing iodine for ten minutes or longer in order to remove mercuric chloride crystals.

Ordinarily, if the PVA film on a slide is thoroughly dried before being stained it adheres perfectly. In rare instances, for reasons not entirely understood, films may wrinkle around the edge or in the middle and show a tendency to slip off the slide during prolonged exposure to aqueous solutions. This tendency may be minimized by preparing relatively thin, rectangular smears. To prevent the loss of a wrinkling film, the slide may be placed in the incubator in a horizontal position and allowed to dry before continuation of the staining process.

In order to test the effect of drying organisms in PVA films, such films were thoroughly dried (over three hours at 37° C.) after each of the different steps in the iron-hematoxylin procedure. After each interruption, the slides were placed in the next solution and the staining was completed in the usual manner. Only those slides were unsatisfactory which were dried after removal from the graded alcohols during dehydration. Therefore, contrary to the usual direction never to allow fecal smears to dry during the staining procedure, in the PVA-fixative technique no particular harm is done at most of the steps.

*Polyvinyl alcohol is marketed under the trade name of Elvanol in various grades representing degrees of polymerization and hydrolysis. The product used in this study was Elvanol 90-25 obtained from E. I. du Pont de Nemours and Co., Electrochemicals Department, Wilmington 98, Del. This does not represent an endorsement of the product by the Public Health Service.

OBSERVATIONS AND DISCUSSION

No differences have been observed between organisms stained after preservation with PVA-fixative and those stained after usual methods of Schaudinn fixation. Nuclear details and cytoplasmic inclusions are clearly defined. Rapid and complete fixation apparently occurs, since many trophozoites exhibit protruding pseudopods.

One distinct advantage of PVA-fixative is that it makes possible successful staining of organisms in liquid specimens. Anyone who has ever attempted to prepare permanent stained mounts of protozoa in cultures, watery stools, or pond water has experienced the difficulty of retaining organisms on the slide during staining procedures. When PVA-fixative is used, it serves to adhere as well as to preserve the organisms and prevents their loss during staining. Stained slides containing many organisms have been made from the following liquid specimens: diarrheic stools and liquid overlay of cultures containing trophozoites of *E. histolytica* (Figs. 1 and 2); loose stools containing trophozoites of *E. coli* (Fig. 3), *Iodamoeba buetschlii* (Fig. 4), *E. nana* (Fig. 5), *D. fragilis* (Fig. 6), and *G. lamblia* (Fig. 7); cultures and pond water containing Paramecium (Fig. 8) and other protozoa; and tap water containing hatched miracidia of *Schistosoma mansoni* (Fig. 9). The only other way known to the authors to stain consistently large numbers of organisms in such specimens is the bulk centrifuge method, employing test tubes. However, the PVA-fixative technique is considerably simpler, particularly when only a few slides are to be prepared.

The technique is also useful for preserving interesting specimens or teaching materials for subsequent staining. A variety of organisms have been preserved in liquid fixative and in dried films for about a year without showing any changes when stained. Of particular interest is a specimen of aspirated material from an amoebic lung abscess preserved in a vial with PVA-fixative (Fig. 10).

At present PVA-fixative cannot be recommended unreservedly for preserving specimens containing cysts. Larger cysts are frequently distorted, apparently during drying of the PVA film. However, in specimens containing many cysts, enough of them with distinct characteristics are usually present to insure correct identification. Occasionally, exceptionally good preparations are obtained even of *E. coli* (Fig. 11) and *I. buetschlii* (Fig. 12) cysts. Smaller cysts such as *G. lamblia* (Fig. 13) and *E. nana* (Fig. 14) are usually not adversely affected. As would be expected, staining PVA-fixative films is not an efficient technique for diagnosing helminth eggs.

In general, stool specimens containing cysts and eggs are best examined in wet mounts prepared either directly from the specimens or following a concentration procedure. In such mounts the refractive appearance of unstained cysts and eggs makes them relatively easy to detect with a low-power objective. Helminth eggs and some cysts usually can be diagnosed in temporary mounts of PVA-fixed specimens, but the fixative destroys the refractiveness of the organisms and renders them more difficult to find and identify.

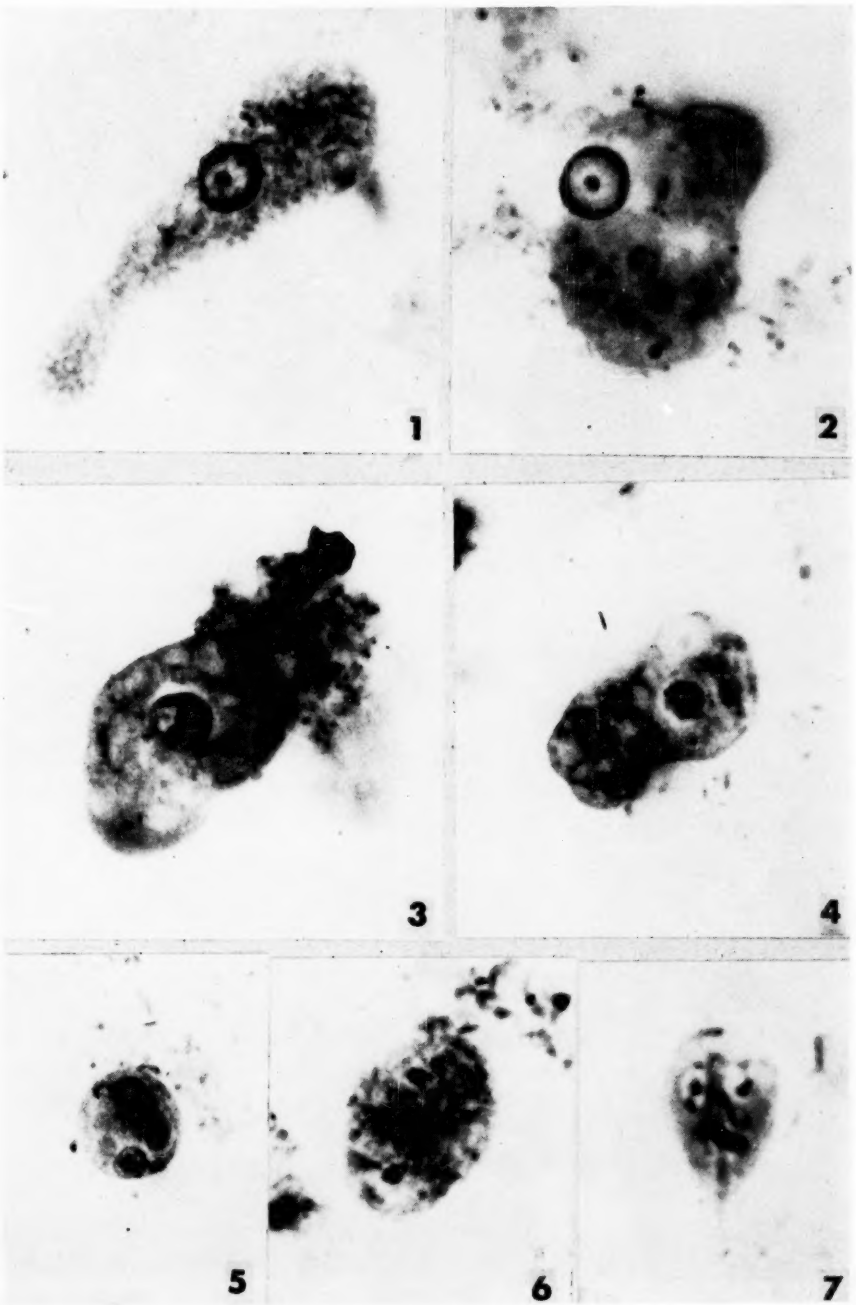

Fig. 1.—*E. histolytica* trophozoite from diarrheic stool. Note protruding pseudopodia.
Fig. 2.—*E. histolytica* trophozoite from liquid overlay of a culture.
Fig. 3.—*E. coli* trophozoite from loose stool.
Fig. 4.—*I. buetschlii* trophozoite from loose stool.
Fig. 5.—*E. nana* trophozoite from loose stool.
Fig. 6.—*D. fragilis* trophozoite from loose stool.
Fig. 7.—*G. iamblia* trophozoite from loose stool.
(All photographs are of organisms stained in PVA-fixative films. Except as indicated films were stained by the long Heidenhain iron-hematoxylin procedure and were photographed at a magnification of 1,125 diameters.)

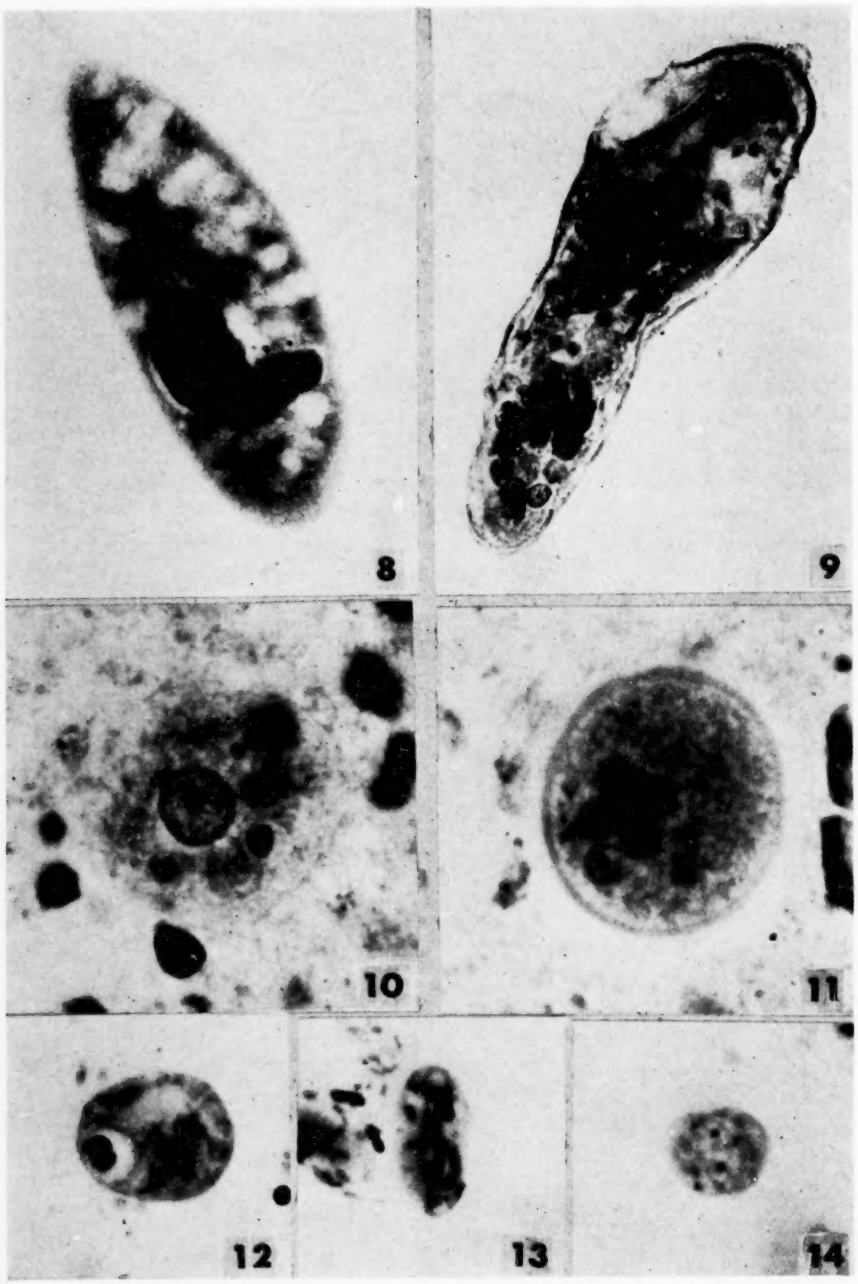

Fig. 8.—*Paramecium* sp. from hay infusion culture. Note micronuclei. (Delafield's hematoxylin, ×500.)
Fig. 9.—*S. mansoni* miracidium from tap water. (Iron-hematoxylin [Goldman, 1949], ×500.)
Fig. 10.—*E. histolytica* trophozoite from lung abscess.
Fig. 11.—*E. coli* cysts from stool. Weigert's copper chrome procedure.
Fig. 12.—*I. buetschlii* cyst from stool.
Fig. 13.—*G. lamblia* cyst from stool.
Fig. 14.—*E. nana* cyst from stool.

(All photographs are of organisms stained in PVA-fixative films. Except as indicated films were stained by the long Heidenhain iron-hematoxylin procedure and were photographed at a magnification of 1,125 diameters.)

Although the PVA-fixative technique might be applied to the study of many different types of organisms, it has been developed primarily to assist the public health laboratory in securing diarrheic, dysenteric, purged, and proctoscopic specimens in a satisfactory condition for diagnosis of amoebiasis. The methods described in this report can be adapted easily to this need. For submitting stool specimens, small vials (15 ml. capacity), two-thirds full of PVA-fixative, can be distributed to physicians in the same manner that vials containing other preservatives are supplied. Since the PVA-fixative technique is not a good method for diagnosing cysts and eggs, the physician should also be supplied with a vial containing no preservative. He should be instructed to place approximately 4 ml. portions of *fresh* liquid or formed stool in each vial and to mix the specimen thoroughly in the vial containing fixative.

With the two-vial method of submitting specimens, a variety of parasitological techniques can be performed and demonstrations of protozoa and helminths can be made regardless of their stage of development. For example, the vial containing PVA-fixative will furnish material for many smears which can be stained and examined for trophozoites and cysts of amoebae and other intestinal protozoa. The vial containing no preservative will furnish material for such gross examinations as consistency of stool, blood, mucus, portions of adult worms, etc.; for microscopic examination of temporary mounts (saline and iodine) for protozoan cysts and helminth eggs and larvae; and for concentration procedures for cysts and eggs.

The expense involved in preparing PVA-fixative is nominal. One pound of PVA costs less than a dollar and is sufficient to make enough fixative to fill a thousand 15 ml. vials approximately two-thirds full. Other ingredients of the fixative—mercuric chloride, ethyl alcohol, acetic acid, and glycerine—are standard laboratory reagents.

SUMMARY

1. Methods are described for the use of polyvinyl alcohol (PVA)-fixative as a preservative for intestinal protozoa and other parasitic and free-living organisms.

2. Organisms so preserved will remain suitable for staining for several months. Best results are obtained with amoebic trophozoites which are otherwise difficult to preserve.

3. In addition to its preservative action, PVA-fixative acts as an adhesive to prevent loss of organisms from smears during staining procedures.

4. The technique has been developed primarily to aid in the diagnosis of amoebiasis and is readily adapted to this need. A two-vial method for shipping stool specimens is suggested which would enable the laboratory to recover all diagnostic stages of the intestinal parasites.

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DESIGN OF A PUMP SUITABLE FOR BLOOD

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THERE has been a need in experimental medicine and therapeutics for a pump that could convey blood under sterile conditions without hemolysis, acceleration of the clotting process, or introduction of pyrogens. Our special interest has been in a pump which will bring the blood of a human subject from a vein to a dialysis apparatus for the removal of products of uremia at variable and appropriate rates. Recently, a number of types of extracorporeal blood dialyzers have been made, some of which utilize pumps of various designs. However, none of these completely satisfies all of the above-mentioned requirements. The various dialyzing procedures for the treatment of uremia, in relation to dietary and other measures, have been the subject of a comprehensive review by Snapper.[1] De Leeuw and Blaustein[2] and Vanatta, Muirhead, and Grollman[3] have noted that the Beck pump, which forms an integral part of the original Kolff "artificial kidney," and serves to return blood from the dialyzer to the vein of the patient, itself produces hemolysis of the blood circulating through it. Vanatta and co-workers have eliminated, therefore, the use of the pump for this purpose and were able to substitute gravity for the return of the blood.

In the following report a simple pump will be described that meets our stringent requirements rather well. In this apparatus there are no valves, close-fitting, or metal parts in contact with the blood. Dead spaces where the current moves slowly are eliminated and air bubbles are not introduced. A self-regulating pressure principle provides safety for the cellophane tubing in the dialysis apparatus proper, and sterility is accomplished by autoclaving.

In principle the pump consists of a Tygon tube of suitable wall thickness and diameter which is compressed in a wave-like motion by a series of twelve keys that push the column of fluid ahead. Behind the crest of the wave, the plastic tubing expands by its own resiliency to fill up again. The baffle plate against which the tubing is compressed is adjusted so that the compression of the tubing is incomplete. Pumping will then cease at a predetermined pressure if increased resistance in the outer circuit causes that pressure to be attained. Furthermore, the compression of the tubing is largely performed by one elevated edge of the key rather than by a flat surface, reducing thereby the area in which mechanical trauma may occur.

In the apparatus (Fig. 1) a constant-speed electric motor with a gear device drives a screw-shaped series of twelve rings on an axle. The rings elevate a series of twelve keys to create pressure on the tubing. The rings are evenly spaced at intervals to form one complete sine wave. The amplitude of the wave can be limited by restricting the extent of motion of the keys. The

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tubing lies between the keys and the adjustable plastic baffle. In usage, the tubing is autoclaved and placed on the keys and the baffle is fastened on top. The baffle can be set for complete compression of the tubing, with the attainment of pressures well over 300 mm. Hg. By loosening the set screws, the baffle can be adjusted to allow leakage at any intermediate pressure.

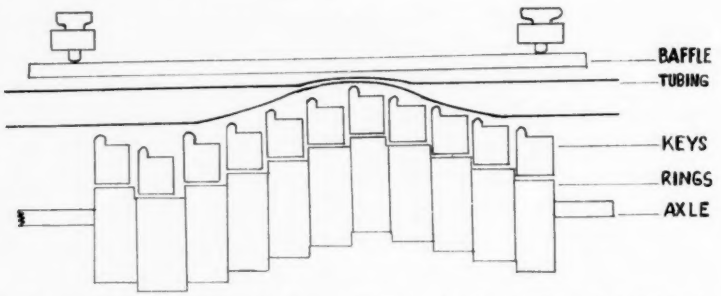

Fig. 1.—Cross-section diagram of pump. Compression incomplete at height of wave.

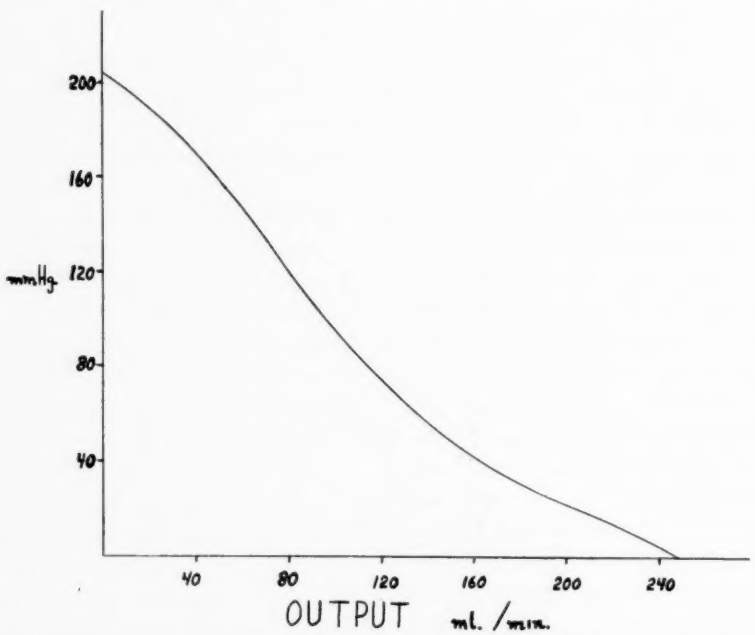

Fig. 2.—Relationship between output and pressure within the system.

In Fig. 2 the inverse relationship between output and pressure within the system is shown in an experiment in which the baffle was regulated to have pumping stop at about 200 mm. Hg. The increase in pressure within the output or arterial side of the system is attained by increased peripheral resistance. Increased resistance on the suction or venous side of the pump would have the same effect of lowering the output of the pump. By limiting the maximal pressure, the cellophane tubing used in dialysis can be safely guarded against undue stress which can result in breakage.

Outputs of from 1 to 18 liters per hour were obtained by varying the stroke volume and the diameter of the tubing used in the model at hand. The output remained constant as long as the peripheral resistance was unchanged.

The pump in the Kolff "artificial kidney" has rollers that create shearing stresses in the tubing and provide a large area for mechanical trauma. In the apparatus described in this report no shearing motion is possible since the keys can move only in the vertical plane. The rate of hemolysis of the blood recirculated through a closed circle of tubing of 20 ml.[3] volume at 37° C. was determined with the pump herein described. Since hemolysis was not observed with normal blood, freshly drawn heparinized blood from a patient in uremia (urea N 148 mg. per cent) was taken for testing. The rate of blood flow was 125 ml. per minute and the maximum pressure attainable was set at 220 mm. Hg. Absolutely no hemolysis was discernible after 12 minutes (72 circuits of the blood). In 18 minutes of recirculation of the blood (108 circuits) a very faint trace and in 24 minutes (144 circuits) a faint trace of hemolysis appeared. Since about 50 circuits of the blood volume through our pump and dialyzer is the maximum recirculation to be expected, the mechanical trauma to the blood by the pump is negligible.

In summary, a pump is described that conveys blood under sterile precautions without hemolysis for 72 circuits of the blood volume. It is without valves, contains no dead space, and provides safety when coupled to a cellophane tube dialyzer by ceasing to pump at predetermined pressures.

The cooperation of Mr. G. J. Frank, Jr., and Mr. J. C. Marsh of the Marsh Laboratory, Pittsburgh, Pa., is gratefully acknowledged.

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AN ELECTROMETRIC METHOD FOR THE DETERMINATION OF RED BLOOD CELL AND PLASMA CHOLINESTERASE ACTIVITY

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THE method for the determination of cholinesterase activity described in this paper was developed to provide a more convenient procedure than those used previously, and one which would require only apparatus generally available in the average laboratory rather than the specialized apparatus heretofore necessary.

The principle of the method is fundamentally the same as that used in all of the chemical methods that have been described and depends upon measurement of the acid produced by the action of cholinesterase on acetylcholine. The acid production is measured in terms of the change in pH produced by enzymatic activity in a standard buffer solution over a definite period of time. The pH is measured with the glass electrode.

The rate of pH change will be a satisfactory measure of enzyme activity if an approximately linear relationship between these two quantities can be established. It is well known that cholinesterase activity decreases markedly with decrease in pH over the range from pH 8 to pH 6, which is approximately the range covered in this method. A buffer has been chosen therefore whose decrease in buffer capacity over this range closely approximates the decrease in enzymatic activity. A further consideration is the possible effect of the added enzyme source (plasma or red cells) on the total buffer capacity of the system. This has been minimized by sufficient dilution of the plasma or red cells so that any buffering effect is low compared with the total buffer present.

The data on which the method is based have been obtained using human blood, but the method is applicable in principle to the blood of other species, and to other tissues, provided that proper allowance is made for the buffer capacity of the material being used.

EXPERIMENTAL

Reagents Required.—

1. Buffer I (for Red Cells): 0.02M sodium barbital (4.1236 Gm.); 0.004M KH_2PO_4 (0.5446 Gm.); 0.60M KCl (44.730 Gm.). For 1 liter of buffer the reagents are dissolved in 900 ml. of distilled water; 28.0 ml. of 0.1N HCl are added while shaking the solution, and the volume is then made to the mark. The pH of buffer I should be 8.10 at 25° C.

2. Buffer II (for Plasma): 0.006M sodium barbital (1.2371 Gm.); 0.001M KH_2PO_4 (0.1361 Gm.); 0.30M NaCl (17.535 Gm.). For 1 liter of buffer dissolve the reagents in about 900 ml. of distilled water and add 11.6 ml. of 0.1N HCl before diluting to volume. The pH of buffer II should be 8.00 at 25° C.

3. Acetylcholine Substrate for Red Cells: 0.11M acetylcholine chloride (2.000 Gm. in 100 ml. of distilled water).

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4. Acetylcholine Substrate for Plasma: 0.165M acetylcholine chloride (3.000 Gm. in 100 ml. of distilled water).

A few drops of toluene are added to all the foregoing solutions as a preservative and the solutions are kept in a refrigerator when not in use.

5. 0.01 per cent saponin.

6. 0.90 per cent NaCl.

These two solutions are made fresh as needed.

Method for Red Cells.—Approximately 5 ml. of freshly drawn blood, containing sufficient heparin to prevent clotting, are centrifuged at 2,000 r.p.m. for fifteen minutes in a graduated centrifuge tube, and the plasma is separated. The cells are mixed with 2 to 3 vol. of 0.9 per cent NaCl and again centrifuged at 2,000 r.p.m. for fifteen minutes. After discarding the supernatant fluid the operation is repeated, centrifuging this time for twenty minutes. The volume of the cells is noted,* and then the saline supernatant is removed to the point where the remaining volume of cells and saline is twice the volume of cells alone. The cells are then mixed thoroughly with the remaining saline. After mixing, 0.4 ml. of cell suspension is hemolyzed in 9.6 ml. of 0.01 per cent saponin solution. One milliliter of hemolyzed red cell solution, representing 0.02 ml. of cells, is added to 1 ml. of buffer solution I and placed in a water bath at 25° C. After allowing ten minutes for equilibration, the initial pH (pH_1) is determined with a pH meter reading to the nearest 0.01 pH unit. Then 0.2 ml. of 0.11M acetylcholine solution is added with rapid mixing and the time is recorded. The enzymatic reaction is allowed to proceed for one to one and one-half hours, and then the final pH of the solution (pH_2) is determined. It is necessary to shake the beaker containing the reaction mixture a few seconds after the electrodes have been immersed to establish rapid equilibrium. The time at which the pH was measured is recorded, and the cholinesterase activity in units of Δ pH/hour is calculated by use of the following formula:

$$\Delta \text{ pH/hour} = \left(\frac{pH_1 - pH_2 - b}{t_2 - t_1} \right) f \qquad \text{(Equation 1)}$$

where pH_1 = initial pH

pH_2 = final pH

t_1 = time of mixing with acetylcholine

t_2 = time of reading pH_2

b = nonenzymatic hydrolysis correction corresponding to pH_2

f = correction for variations in Δ pH/hour with pH, corresponding to pH_2

The b and f corrections are given in Table I.

TABLE I. CORRECTION FACTORS FOR USE IN EQUATION 1

	RED CELL CHOLINESTERASE CORRECTIONS		PLASMA CHOLINESTERASE CORRECTIONS	
pH_2	b	f	b	f
7.9	0.03	0.94	0.09	0.98
7.8	0.02	0.95	0.07	1.00
7.7	0.01	0.96	0.06	1.01
7.6	0.00	0.97	0.05	1.02
7.5	0.00	0.98	0.04	1.02
7.4	0.00	0.99	0.03	1.01
7.3	0.00	1.00	0.02	1.01
7.2	0.00	1.00	0.02	1.00
7.1	0.00	1.00	0.02	1.00
7.0	0.00	1.00	0.01	1.00
6.8	0.00	0.99	0.01	1.00
6.6	0.00	0.97	0.01	1.01
6.4	0.00	0.97	0.01	1.02
6.2	0.00	0.97	0.01	1.04
6.0	0.00	0.99	0.01	1.09

*This volume is dependent in part upon the centrifugal force and the time during which it is applied. The average centrifugal force used in this work was approximately 800 times gravity.

To express results in terms of per cent activity relative to some normal value, multiply the Δ pH/hour value found for the sample by 100 and divide by the Δ pH/hour value considered to be (or found to be) normal. For example, the average Δ pH/hour value for red cell cholinesterase activity in twelve normal human subjects has been found to be 0.753. If an unknown sample gave a value of say 0.550, then the per cent activity would be $\frac{0.550}{0.753} \times 100 = 73$ per cent.

The red cell buffer (buffer I) is made to give a pH of 8.00 after mixing with diluted red cells. Sodium barbital has its maximum buffer capacity in the region of pH 8.0[1] and this is also the region of optimal cholinesterase activity.* With the ratio of buffer to red cells used, the decrease in buffer capacity parallels the decrease in enzyme activity with decreasing pH to give an almost constant value for the enzyme activity in Δ pH/hour. This is shown by the relatively small deviations from unity of the *f* factors given in Table I. Potassium dihydrogen phosphate is added to maintain the nearly constant relationship down to pH 6.00. The buffer capacity contributed by the added red cells averages 12 per cent of the total buffer capacity, determined by titration between pH 8.00 and pH 7.00.

The activating effect of potassium chloride on red cell cholinesterase has been described by Mendel and Rudney[3] and Augustinsson.[2] This salt was used in preference to the magnesium or calcium salts customarily employed as activators to avoid precipitation of phosphate. The concentration employed was found to be in the optimum range for activation. Hemolysis by saponin prevents the precipitation of the red cell stroma by the potassium chloride in the buffer.

Although the substrate concentration is greater than the optimum reported for mammalian red cell cholinesterase by Augustinsson,[2] it was necessary to use a final concentration of 0.01M acetylcholine in order to maintain the nearly constant rate of pH change over the selected pH range.

Method for Plasma.—The plasma is diluted with water so that each milliliter of solution contains 0.02 ml. plasma. One milliliter of diluted plasma is mixed with 1 ml. of buffer solution II. The solution is allowed to equilibrate in a water bath at 25° C. for ten minutes, and then 0.2 ml. of 0.165M acetylcholine is added with mixing. The subsequent determination is carried out, and the activity calculated, in the same manner as described for red cells, using the correction values for plasma given in Table I.

Human plasma contains less cholinesterace activity and has less buffer capacity than the equivalent volume of red cells. Therefore, a more dilute buffer is used. However, similar considerations apply to the decrease in enzyme activity with decreasing pH, as discussed in the preceding section. The buffer capacity of the plasma averages 7 per cent of the total. Salts have a relatively small effect on the activity of human plasma cholinesterase,[4] but the pH of the dilute buffer used can be affected by changes in salt concentration.[5] Sodium chloride is added to the buffer to reduce possible variations in total salt concentration.

Both buffers I and II slowly decrease in pH and buffer capacity after several weeks and should be checked with a pH meter at the time they are used. If the pH_1 reading for a determination is more than 0.03 pH units below 8.00, a fresh buffer solution should be prepared.

Pure acetylcholine salts will produce a pH change of less than 0.01 pH unit when added to buffers I or II in the concentrations specified in the Methods sections.

Comparison of the Electrometric Method With the Manometric Method.—Cholinesterase was determined on thirty-one independent samples of plasma and twenty-seven independent samples of red cells from twelve male human subjects, using both the electrometric method described here and a modified form of the manometric method of Ammon.[6] In the manometric procedure the reaction mixture for plasma cholinesterase contained 0.05 ml. of plasma and 3.0 ml. of 0.025M sodium bicarbonate. For determination of red cell cholinesterase

*See reference 2 for bibliography.

each Warburg manometric vessel contained 0.02 ml. of cells and 3.0 ml. of 0.025M sodium bicarbonate containing 0.0342M magnesium chloride. In each instance the substrate was recrystallized acetylcholine bromide in a final concentration of 0.015M. The enzyme was tipped from the side arm after ten minutes of equilibration with 5 per cent carbon dioxide in nitrogen. Manometric readings were taken over a forty-minute period, the temperature being maintained at 38° C. The electrometric procedure was carried out as described here, the temperature of the reaction being 25° C.

The mean value of the cholinesterase activity by the electrometric method was 0.703 Δ pH per hour for plasma and 0.753 Δ pH per hour for red cells. The corresponding values by the manometric method were 226.0 microliters per hour for plasma and 253.0 microliters per hour for red cells. Relative activities were calculated for both methods by dividing individual activities by the appropriate mean values. The differences between the relative activities determined by the electrometric method and the corresponding relative activities by the manometric method were calculated. The standard deviation of the differences was 5.49 per cent for plasma cholinesterase and 5.50 per cent for red cell cholinesterase.

Nonenzymatic Hydrolysis Correction.—The nonenzymatic hydrolysis correction, *b*, was determined by measuring the pH change with time in solutions containing buffer, acetylcholine, and inhibited cholinesterase. Diisopropyl fluorophosphite (DFP) (10 μg per milliliter of diluted cells or plasma) was used to inhibit the cholinesterase. The pH was determined at intervals of several hours and *b* was then calculated by subtracting the observed pH from 8.00 and dividing by the time in hours.

A slow lowering of pH occurs in the solution of the buffer and enzyme in the absence of substrate and is included in the *b* correction.

Correction Factor f.—The values for the correction *f*, given in Table I, are based on samples taken from ten subjects. To determine the values, activity measurements were made as described in the Methods section, but pH readings were taken successively on each sample at approximately ten-minute intervals over the pH range from 8.00 to 6.00. The value of *f* at pH 7.00 was arbitrarily set equal to 1.00. Using Equation 1, the values of *f* were calculated for the selected pH range.

DISCUSSION

The advantages of the electrometric pH method are simplicity, a requirement for a minimum of equipment, and the possibility of doing a large number of determinations in a relatively short time. The pH readings may be made easily in a time of one minute for each sample. It is therefore possible to do a large series of determinations in a few hours by adding the substrate to the samples at one-minute intervals.

After this method had been developed, a similar procedure by Croxatto and associates[7] was found in which rate of pH change is used as a measure of serum cholinesterase activity, the pH being measured colorimetrically in a Pulfrich photometer. This method is applicable only to relatively uncolored solutions.

SUMMARY

1. An electrometric method for plasma and red cell cholinesterase activity is presented in which the enzymatic release of acetic acid from acetylcholine is measured in units of pH change per hour, in a solution of standard buffer capacity.

2. The method has been compared with the standard manometric procedure. The standard deviation of the differences between the methods was 5.49 per cent for plasma cholinesterase and 5.50 per cent for red blood cell cholinesterase.

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STERILIZATION OF DEFUNCTIONALIZED LOOPS OF COLON IN PREPARATION FOR ANASTOMOSIS WITH OTHER VISCERA

A Method of Study and Appropriate Selection of Antibacterial Agents

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IN ADMINISTERING intestinal antiseptics for surgery of the large bowel a reduction of the bacterial count of the feces, timed to be at a maximum on the day of operation, is desirable. Two patients were recently treated with a combination of antibiotic and chemotherapeutic agents to prepare the colon for anastomosis with other viscera. At operation full thickness segments of colon approximately 1.0 cm. square were excised from the operative site for aerobic and anaerobic bacteriologic cultures. In the first case the cultures revealed no growth. Forty-eight hour cultures from the second case were sterile, but at the end of seventy-two hours rare colonies of *Monilia albicans* and diphtheroid bacilli were recovered. All organisms in the pretreatment cultures of colonic contents, which showed the usual profuse mixed intestinal flora, were absent (Table I).

The first patient (R. I.) was a 61-year-old man with recurrent obstructing carcinoma of the esophagus following a previous transthoracic resection at which time an extremely short jejunal mesentery was noted. After preliminary gastrostomy for the obstruction, a right transverse colostomy was done to facilitate the preparation of his bowel. On April 13, 1949, Dr. R. H. Smithwick did a transthoracic resection of the recurrent carcinoma and because the short jejunal mesentery precluded an esophagojejunostomy, the transverse colon was used for an esophagocolostomy. At a second stage the continuity of the gastrointestinal tract was re-established, leaving a transplanted segment of colon within the thoracic cavity.

The second patient (W. O.) was a 64-year-old man with carcinoma of the anterior wall of the rectum. During a preliminary cystoscopy the instrument was inadvertently passed through the posterior urethra into the rectum through the carcinomatous mass. Because of this a transverse colostomy was done the same day and it later proved useful in preparing the bowel for surgery. On May 26, 1949, a resection of the rectum, bladder, prostate, seminal vesicles, and posterior urethra with uretero-intestinal anastomosis was done.

Both of these cases will be reported fully at a later date, but the unexpected bacteriologic findings have prompted this preliminary report on the method used for study and preparation of the bowel.

PLAN OF STUDY AND ANTIBACTERIAL TREATMENT

Therapy was planned to secure reduction in the flora of the colon, timed to be maximal on the day of operation. The choice of antibiotic agents was guided by the sensitivity of the intestinal flora in each instance. Cultures taken just before operation were used to work out the sensitivities to various antibiotics of the organisms remaining in the colon as changed by the intes-

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TABLE I. PREOPERATIVE AND POSTOPERATIVE CULTURES, INTESTINAL FLORA, AND COLIFORM COUNTS

	ORGANISMS	INITIAL CULTURE	PREOPERATIVE CULTURE	FINAL CULTURE (EXCISED COLON TISSUE)
Case 1 (Patient R. I.)	Staphylococcus albus	+	0	0
	Diphtheroid bacilli	+	+	0
	Nonhemolytic streptococcus	+	+	0
	Alpha hemolytic streptococcus	+	+	0
	Bacillus mucosus capsulatus	+	0	0
	Escherichia coli	+	0	0
	Aerobacter aerogenes	+	+	0
	Bacillus pyocyaneus	+	+	0
	Preoperative day	10 9 8 7	6 5 4	3 2 1
	Coliform count/gram of feces	10,000		0 0 0
Case 2 (Patient W. O.)	Staphylococcus albus	+	+	0
	Alpha hemolytic streptococcus	+	0	0
	Alpha hemolytic streptococcus (enterococcus type)	+	+	0
	Bacillus pyocyaneus	+	+	0
	Escherichia coli	+	0	0
	Proteus vulgaris	+	0	0
	Bacillus mucosus capsulatus	+	0	0
	Monilia albicans		0	+
	Diphtheroid bacilli		0	+
	Bacillus subtilis		+	0
	Preoperative day	13 12 10 9	8 7 6	3 2 0
	Coliform count/gram of feces	10,000 12,000	30,000	5,500 0

tinal antiseptic therapy. Armed with this information in advance, it should be possible to give systemic or topical treatment with greater speed and specificity for an infection which might develop from the colon after operation. Because of the rapid development of resistance of certain organisms to streptomycin, it was decided to refrain from using it as an intestinal antiseptic but to reserve it for later use in case infection developed.

Cultures of colon contents were obtained through the distal limb of the colostomy. A sterile rubber tube was inserted as far into the bowel as possible and a long slender pipette passed through this tube was used to aspirate colonic juice. All cultures were planted on blood agar plates and inoculated into 6.0 c.c. of beef heart infusion broth enriched with sheeps' blood and fortified with 5.0 mg. per cent of para-aminobenzoic acid, each inoculation consisting of a 2 mm. platinum wire loopful of the juice. Tubes of chopped meat media fortified with infusion broth and sealed with petroleum jelly and paraffin were used for anaerobic cultures. Coliform counts were done by a modification of the streak method described by Poth.[1]

Cultures of feces were taken on the day the colostomies were opened and are shown in Table I. Colostomy and rectal enemas and instillations of 25 per cent magnesium sulfate served to clean out the isolated loops of colon. Patients were given a low residue diet and vitamin K parenterally.

In order to get a rough idea of the sensitivity of the intestinal flora upon which to base antibiotic therapy, blood agar plates were inoculated with swab cultures of the feces and tested by a filter paper disk contact method for sensitivity to aureomycin and chloromycetin.*

*A discussion of this method will be the subject of a separate report.

Twenty-four hour broth cultures diluted 10^{-6} were also tested by a serial dilution technique against these same two antibiotics with the idea of using the most appropriate one as indicated by these sensitivities. The chosen antibiotic was given together with Sulfathaladine as an intestinal antiseptic. It was hoped that this combination would be effective against both gram-negative and gram-positive organisms. The results of these and the subsequent sensitivity tests are indicated in Table II. In the presence of an enterostomy or diarrhea or when enemas or purgatives are to be used, Sulfathaladine is preferable to Sulfasuxidine because of its greater bacteriostatic activity[1] and therefore it was chosen in these cases. For the first patient (R. I.) chloromycetin was selected rather than aureomycin because, although the flora was within range of sensitivity to both antibiotics, aureomycin would have been more likely to cause nausea and the patient was in precarious fluid and electrolyte balance. Inasmuch as chloromycetin was also within the effective range of sensitivity, it was selected to be given with the Sulfathaladine. In the second patient (W. O.) the flora was within the range of sensitivity to both antibiotics so aureomycin was selected because it was more readily available. Administration of the chosen agents was then started via all available routes. Large doses were given as colostomy and rectal irrigations, and also, because of the possibility of "spill over" contamination from the proximal colostomy loop, these drugs were given through the gastrostomy and by mouth as well. Table III indicates the dosage and routes of administration of the various agents.

On the third day before operation, cultures from the colonic juice were obtained to work out the sensitivities of each remaining organism as changed by a period of therapy in order to better cope with infection, should it occur (Table II).

On the second or first preoperative day it was desired to administer prophylactic penicillin and since Poth and co-workers[2] have shown a definite penicillin-Sulfathaladine antagonism affecting certain gram-negative organisms, the Sulfathaladine was discontinued and Sulfasuxidine was substituted. This allowed the unimpeded action of penicillin for the last one or two days before operation after having taken advantage of the superior action of Sulfathaladine during the major part of the preparation. Intramuscular penicillin was then started, and to the first patient (R. I.) penicillin aerosol and lozenges also were given in an attempt to cut down the gram-positive flora in the nasopharynx, throat, and esophagus.

The daily dose of Sulfathaladine and Sulfasuxidine was crushed and suspended in saline in which the antibiotic had been dissolved. This was then instilled into the colostomies, gastrostomy, and rectum in equally divided doses at 6 and 10 A.M. and 2, 6, and 10 P.M., the volume of each instillation being about 100 to 200 cubic centimeters. The last day's dose was dispensed in a very small volume (10 c.c. for each instillation) so that the bowel would be dry at operation. Oral medications were given in equally divided doses on the same schedule.

The following is a summary of the plan of study and treatment in outline form.

1. Reserve streptomycin for later use if infection should develop.

2. After the colostomy is open, culture the transverse colon. Test the mixed cultures for sensitivity against available antibiotics suitable for oral administration.

3. Give colostomy and rectal enemas and magnesium sulfate instillations until the bowel is clean; then use appropriate antibiotic agent along with Sulfathaladine for instillations into the bowel.

4. Three days before operation, culture the mid-colon and work out sensitivities of the constituents of the flora as now altered by therapy to various antibiotics.

5. Two days before operation, omit Sulfathaladine and substitute Sulfasuxidine. Start intramuscular penicillin, penicillin aerosol, and penicillin lozenges.

TABLE II. SENSITIVITY TEST RESULTS

CASE	ANTIBACTERIAL AGENT TESTED		INITIAL MIXED CULTURE		PEROPERATIVE CULTURE—INDIVIDUAL CONSTITUENTS							
					SERIAL DILUTION METHOD				FILTER PAPER DISK METHOD			
			SERIAL DILUTION METHOD	FILTER PAPER DISK METHOD*	B. PYOCYANEUS	A. AEROGENES	NONHEMOLYTIC STREPTOCOCCUS	ALPHA HEMOLYTIC STREPTOCOCCUS	B. PYOCYANEUS	A. AEROGENES	NON-HEMOLYTIC STREPTOCOCCUS	ALPHA HEMOLYTIC STREPTOCOCCUS
1 (R. I.)	Aureomycin	γ/ml.	1.5	250-500	10	3	30	0.1	500-1,000	250-500	125-250	6-125
	Chloromycetin	γ/ml.	6.25	50	25	6.25	6.25	6.25	50	50-100	50-100	50-100
	Streptomycin	U/ml.			15	7.5	15	7.5	0-20	0-20	250-500	100-500
	Penicillin	U/ml.			1,000	250	1.5	3.5	----	1,000	20-100	0-20
	Bacitracin	U/ml.			1,000	125	>0.3	>0.3	>1,000	500-1,000	0-20	0-20
	Sulfamylon	%			0.3	0.6	0.15	0.07	0.06	1.25-2.5	0-0.6	0-0.6
	Urethane	%			5.0	5.0	5.0	5.0	20	20	20	----
	Urethane	%			2.5	2.5	0.01	0.007	10	10	0-1.25	0-0.6
	Penicillin	U/ml.			250	250	1.25	0.7	1,000	1,000	0-125	0-20
	Streptomycin	U/ml.			1.5	6.0	3.0	1.5	0-25	0-20	0-25	25-50
	Sulfamylon	%			0.03	0.15	0.07	0.003	0-0.6	0-0.6	0-0.6	0.6-1.25
					STAPH. ALBUS	ALPHA STREPTOCOCCUS	B. SUBTILIS	ESCH. COLI	STAPH. ALBUS	ALPHA STREPTOCOCCUS	B. SUBTILIS	ESCH. COLI
2 (W. O.)	Aureomycin	γ/ml.	5	125-250	0.7	10	0.1	7	0-10	250-500	0-10	500-1,000
	Chloromycetin	γ/ml.	3.15	50-100	6.25	3.15	3.15	25	25-50	50-100	0-12.5	25-50
	Streptomycin	U/ml.			15	63	32	125	20-100	1,000	100-500	20-100
	Penicillin	U/ml.			0.15	3.5	0.07	1,000	0-2	20-100	0-20	1,000
	Bacitracin	U/ml.			0.3	0.7	12.5	500	0-20	20-100	100-500	1,000
	Sulfamylon	%			0.07	0.01	0.007	2.5	0-0.6	0-0.6	0-0.6	2.5-5
	Urethane	%			5	5	2.5	5	25-50	25-50	12.5-25	25-50
	Urethane	%			0.003	5	0.0015	5	0-1.25	0-1.25	0-1.25	10
	Penicillin	U/ml.			0.3	500	0.15	500	0-125	0.125	0-125	1,000
	Streptomycin	U/ml.			3	0.3	0.15	100	0-25	0-25	0-25	50-100
	Sulfamylon	%			0.07	0.007	0.03	2.5	0-0.6	0-0.6	0-0.6	1.25-2.5

*Direct swab inoculation onto blood agar plate. Ten minute contact with disk wet with testing solution. No preliminary incubation. Results in two figures indicate the concentration that bracketed the actual sensitivity which lies somewhere between them.

TABLE III. DAILY DOSAGE AND ROUTES OF ADMINISTRATION OF ANTIBACTERIAL AGENTS

TABLE III. DAILY DOSAGE AND ROUTES OF ADMINISTRATION OF ANTIBACTERIAL AGENTS

PREOPERATIVE DAY			9	8	7	6	5	4	3	2	1	DAY OF OPERATION
By mouth or gastrostomy	Case 1 (R. I.)	Sulfathaladine (Gm.)	5	5	5	5	5	5	5	0	0	0
		Sulfasuxidine (Gm.)								10	10	0
		Chloromycetin (Gm.)	1	1	1	1	1	1	1	1	1	0
	Case 2 (W. O.)	Sulfathaladine (Gm.)							5	5†	5†	0
		Sulfasuxidine (Gm.)	8*	8*	8*	‡	‡	‡				0
		Aureomycin (Gm.)							2.5	2.5	2.5	0
Penicillin	Case 1 (R. I.)	Crysticillin (units)										300,000
		Penicillin (units)										300,000
		Penicillin aerosol (units)								90,000	90,000	0
		Penicillin troches (units)								1,500	1,500	0
	Case 2 (W. O.)	Crysticillin (units)	600,000*	600,000*	600,000*						300,000	300,000
		Penicillin (units)										600,000
Instilled into colostomy or rectum	Case 1 (R. I.)	Sulfathaladine (Gm.)	8	8	8	8	8	8	8			0
		Sulfasuxidine (Gm.)								15	15	0
		Chloromycetin (Gm.)	2	2	2	2	2	2	2	2	2	0
	Case 2 (W. O.)	Sulfathaladine (Gm.)				8	8	8	8	8	0	0
		Sulfasuxidine (Gm.)	12*	12*	12*						12	0
		Aureomycin (Gm.)	3*	3*	3*	3	3	3	3	3	3	0

*Case 2 (Patient W. O.) was being treated with aureomycin, Sulfasuxidine, and procaine penicillin (crysticillin) for the urethral perforation before the sixth preoperative day when the regime for preparation of the bowel as outlined in the text was begun.

†Given by error.

‡Oral medication omitted because of marked skin irritation around colostomy.

TABLE II. SENSITIVITY TEST RESULTS

CASE	ANTIBACTERIAL AGENT TESTED		INITIAL MIXED CULTURE		PEROPERATIVE CULTURE—INDIVIDUAL CONSTITUENTS							
					SERIAL DILUTION METHOD				FILTER PAPER DISK METHOD			
			SERIAL DILUTION METHOD	FILTER PAPER DISK METHOD*	B. PYOCYANEUS	A. AEROGENES	NONHEMOLYTIC STREPTOCOCCUS	ALPHA HEMOLYTIC STREPTOCOCCUS	B. PYOCYANEUS	A. AEROGENES	NON-HEMOLYTIC STREPTOCOCCUS	ALPHA HEMOLYTIC STREPTOCOCCUS
1 (R. I.)	Aureomycin	γ/ml.	1.5	250-500	10	3	30	0.1	500-1,000	250-500	125-250	6-125
	Chloromycetin	γ/ml.	6.25	50	25	6.25	6.25	6.25	50	50-100	50-100	50-100
	Streptomycin	U/ml.			15	7.5	15	7.5	0-20	0-20	250-500	100-500
	Penicillin	U/ml.			1,000	250	1.5	3.5	----	1,000	20-100	0-20
	Bacitracin	U/ml.			1,000	125	>0.3	>0.3	>1,000	500-1,000	0-20	0-20
	Sulfamylon	%			0.3	0.6	0.15	0.07	0.06	1.25-2.5	0-0.6	0-0.6
	Urethane	%			5.0	5.0	5.0	5.0	20	20	20	----
	Urethane	%			2.5	2.5	0.01	0.007	10	10	0-1.25	0-0.6
	Penicillin	U/ml.			250	250	1.25	0.7	1,000	1,000	0-125	0-20
	Streptomycin	U/ml.			1.5	6.0	3.0	1.5	0-25	0-20	0-25	25-50
	Sulfamylon	%			0.03	0.15	0.07	0.003	0-0.6	0-0.6	0-0.6	0.6-1.25
					STAPH. ALBUS	ALPHA STREPTOCOCCUS	B. SUBTILIS	ESCH. COLI	STAPH. ALBUS	ALPHA STREPTOCOCCUS	B. SUBTILIS	ESCH. COLI
2 (W. O.)	Aureomycin	γ/ml.	5	125-250	0.7	10	0.1	7	0-10	250-500	0-10	500-1,000
	Chloromycetin	γ/ml.	3.15	50-100	6.25	3.15	3.15	25	25-50	50-100	0-12.5	25-50
	Streptomycin	U/ml.			15	63	32	125	20-100	1,000	100-500	20-100
	Penicillin	U/ml.			0.15	3.5	0.07	1,000	0-2	20-100	0-20	1,000
	Bacitracin	U/ml.			0.3	0.7	12.5	500	0-20	20-100	100-500	1,000
	Sulfamylon	%			0.07	0.01	0.007	2.5	0-0.6	0-0.6	0-0.6	2.5-5
	Urethane	%			5	5	2.5	5	25-50	25-50	12.5-25	25-50
	Urethane	%			0.003	5	0.0015	5	0-1.25	0-1.25	0-1.25	10
	Penicillin	U/ml.			0.3	500	0.15	500	0-125	0.125	0-125	1,000
	Streptomycin	U/ml.			3	0.3	0.15	100	0-25	0-25	0-25	50-100
	Sulfamylon	%			0.07	0.007	0.03	2.5	0-0.6	0-0.6	0-0.6	1.25-2.5

*Direct swab inoculation onto blood agar plate. Ten minute contact with disk wet with testing solution. No preliminary incubation. Results in two figures indicate the concentration that bracketed the actual sensitivity which lies somewhere between them.

TABLE III. DAILY DOSAGE AND ROUTES OF ADMINISTRATION OF ANTIBACTERIAL AGENTS

TABLE III. DAILY DOSAGE AND ROUTES OF ADMINISTRATION OF ANTIBACTERIAL AGENTS

PREOPERATIVE DAY			9	8	7	6	5	4	3	2	1	DAY OF OPERATION
By mouth or gastrostomy	Case 1 (R. I.)	Sulfathaladine (Gm.)	5	5	5	5	5	5	5	0	0	0
		Sulfasuxidine (Gm.)								10	10	0
		Chloromycetin (Gm.)	1	1	1	1	1	1	1	1	1	0
	Case 2 (W. O.)	Sulfathaladine (Gm.)							5	5†	5†	0
		Sulfasuxidine (Gm.)	8*	8*	8*	‡	‡	‡				0
		Aureomycin (Gm.)							2.5	2.5	2.5	0
Penicillin	Case 1 (R. I.)	Crysticillin (units)										300,000
		Penicillin (units)										300,000
		Penicillin aerosol (units)								90,000	90,000	0
		Penicillin troches (units)								1,500	1,500	0
	Case 2 (W. O.)	Crysticillin (units)	600,000*	600,000*	600,000*						300,000	300,000
		Penicillin (units)										600,000
Instilled into colostomy or rectum	Case 1 (R. I.)	Sulfathaladine (Gm.)	8	8	8	8	8	8	8			0
		Sulfasuxidine (Gm.)								15	15	0
		Chloromycetin (Gm.)	2	2	2	2	2	2	2	2	2	0
	Case 2 (W. O.)	Sulfathaladine (Gm.)				8	8	8	8	8	0	0
		Sulfasuxidine (Gm.)	12*	12*	12*						12	0
		Aureomycin (Gm.)	3*	3*	3*	3	3	3	3	3	3	0

*Case 2 (Patient W. O.) was being treated with aureomycin, Sulfasuxidine, and procaine penicillin (crysticillin) for the urethral perforation before the sixth preoperative day when the regime for preparation of the bowel as outlined in the text was begun.

†Given by error.

‡Oral medication omitted because of marked skin irritation around colostomy.

DISCUSSION

When a culture is taken from a bowel being prepared with intestinal antiseptics, some of the antiseptic agent is transferred to the media along with the bacteria and might prevent growth of viable organisms. This is particularly true on solid media and perhaps less so in liquid media. The cultures referred to in Table II are broth cultures and since the media contained para-aminobenzoic acid which inhibits the sulfonamides, it is unlikely that the small amounts of Sulfathaladine or Sulfasuxidine transferred to the broth had any bactericidal action. With the doses of chloromycetin and aureomycin employed, it would seem likely that these antibiotics were diluted beyond their effective bactericidal concentration by the broth. Objections grounded on the transfer of antibacterial agents to the media are largely theoretical because contamination in the operative field would likewise transfer such bactericidal amounts of these agents. It would therefore appear that a practical if not absolute sterilization of the colon has been achieved. It is possible that the appearance of rare colonies of *Monilia albicans* and diphtheroids in the seventy-two hour culture of the second case represents contamination inasmuch as the bacteria did not grow in the original cultures. It is hoped that this type of preparation of the bowel may further reduce the risk of infection and perhaps extend the scope of the surgical usefulness of the colon.

When tested by the serial dilution technique, the mixed flora in the cases reported here were sensitive to concentrations of chloromycetin and aureomycin ranging from 1.5 gamma per cubic centimeter to 6.25 gamma per cubic centimeter. However, preliminary data being collected by Hewitt[3] would seem to indicate that some organisms commonly encountered in the gastrointestinal tract are resistant to much higher levels, especially the Pseudomonas group in which many strains are resistant to 50 gamma per cubic centimeter or more. The effective sensitivities for aureomycin are more difficult to determine because this drug is quite unstable and is affected by a variety of substances and media.[4] With a twenty-four hour serial dilution sensitivity technique the values seem to approximate those of chloromycetin, the Pseudomonas again being relatively resistant.

The technique described for taking cultures from the colon with a pipette passed through a rubber tube is not satisfactory. The apparatus cannot be manipulated far enough into the colon and contaminants are carried inward from the presenting stoma of the colostomy. It is probable that the antibacterial effect deep within the limb of the colon where there is puddling of the solution is greater than in the segment than can be reached by this technique. Other methods are being studied.

Because no infection developed in these patients it was not necessary to use the information gained from the preoperative cultures and sensitivity studies. It is believed that such advance information would make possible a more rapid and intelligent choice of antibacterial agents. This is especially true in extensive operations involving potential contamination of areas par-

ticularly vulnerable to infection, such as retroperitoneal spaces and the thoracic cavity, from which cultures cannot be obtained postoperatively until infection has been established and drained.

CONCLUSIONS

A plan of study and antibacterial treatment for preparation of the colon for surgery is described.

In one patient thus prepared, a seventy-two hour broth culture of an excised segment of colon taken from the operative site yielded no growth. In a second patient, rare colonies of *Monilia albicans* and diphtheroids were recovered, but all of the original intestinal organisms were absent.

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A TECHNIQUE FOR THE COLLECTION OF LYMPH FROM THE RIGHT THORACIC DUCT IN DOGS

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ST. LOUIS, MO.

STUDIES of experimental pulmonary edema have been frequently handicapped by the lack of a method for the detection of edema in intact and functioning lungs, particularly when the transudation was occult or of low intensity. In their studies of pulmonary lymph, Drinker and associates[1, 2] noted that the flow of lymph from the lungs was increased when congestion was induced. We have recently investigated the outflow of lymph from the lungs in relation to congestion and edema of the pulmonary tissue from experimental heart failure.[3] These observations have indicated that the production of lung lymph is always increased with pulmonary engorgement and edema, and that the quantity of flow is greater with more intense grades of transudation.

It has been shown[1] that the pulmonary and cardiac lymph passes through lymph nodes lying along the superior vena cava. The fluid then courses from these nodes to the right subclavian vein through one or more lymphatics comprising the *right thoracic duct.* In some animals, the pulmonary lymphatics pass to the left, anastomosing with the thoracic duct or entering directly into the left subclavian vein. Drinker and co-workers[1] have collected the pulmonary lymph by cannulation of the right thoracic duct. However, the procedure was so difficult as to be "accomplished satisfactorily in about one out of five animals." In our experience, the cannulation technique has had serious drawbacks. In many animals, the lymphatics were found to be too small to cannulate. In others, cannulation was successful but the lymph failed to flow because of mechanical obstruction by the cannula.

We have employed a simple method for the collection and measurement of right thoracic duct lymph which avoids the pitfalls of minute lymphatics and possible artificial obstruction of the vessels by the manipulation.

METHOD

The procedure is carried out on dogs under Nembutal anesthesia. To facilitate identification of the lymphatic, 10 c.c. of 1 per cent solution of Evans blue is instilled into the lower part of the trachea through a glass tube. This tube may be introduced through a tracheotomy or through the glottis. A period of ten minutes is allowed for the absorption of the dye into the pulmonary lymphatics. A longitudinal incision is made along

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the right external jugular vein extending to the pectoral muscles and the first rib. The lower end of the jugular vein is then dissected free and followed to its confluence with the axillary vein and the right subclavian vein[4] (see Fig. 1). The subclavian vein, in its mid-portion, is in close apposition to the subclavian artery which arches up from beneath. Although there is considerable variation in the right lymphatic vessels, one or more lymphatics have been found in this area constantly, lying either across the ventral surface of the subclavian vein or between the artery and vein, arching upward from the deeper tissue. If dissection is first carried out in this field, location of the lymphatics requires a very short time; a tedious dissection is usually not necessary.

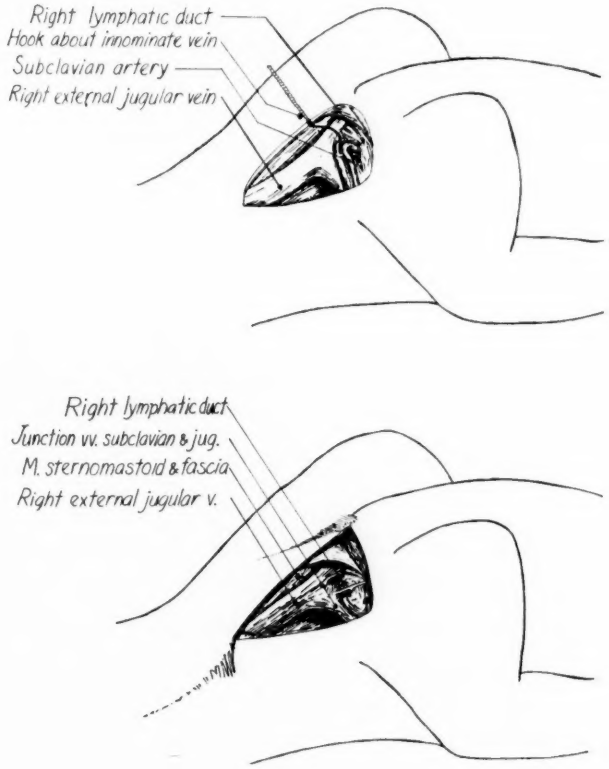

Fig. 1.

Collection of the lung lymph is accomplished by incising the vessel with fine scissors, permitting the fluid to issue freely. The field is carefully wiped dry, and the lymph is then caught on small pledgets of absorbent cotton (10 to 20 mg. in weight) placed over the incised vessel for a measured time. In the properly executed dissection, where all bleeding has been controlled, the lymph is the only fluid absorbed by the cotton. Furthermore, the identity of the lymph is assured by the bluish stain from the dye. We employ a Roller-Smith precision balance to facilitate rapid and accurate weighing of the pledgets.

In normal anesthetized dogs, our method shows that the quantity of lymph from a right thoracic lymphatic vessel varies from 4 to 50 mg. a minute. Drinker and co-workers,[1, 2] by cannulation of the lymphatic, find the flow to range from 3.7 to 59.4 mg. a minute. While cannulation is extremely difficult, being unsuccessful in four out of five animals, the pledget technique in all but rare instances is easily accomplished.

SUMMARY

Anatomic landmarks which facilitate the location of the right thoracic duct are described. By incision of the lymphatic vessel, the fluid is collected on weighed cotton pledgets for controlled periods. The method has proved to be accurate and simple to carry out.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

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ABSTRACTS

1. THE DYNAMICS OF COAGULATION

J. Garrott Allen, M.D., Peter V. Moulder, M.D. (By Invitation), Daniel M. Enerson, M.D. (By Invitation), and Donald Glotzer, B.S. (By Invitation), Chicago, Ill.

Spontaneous (?) bleeding in the presence of hemophilia, prothrombin deficiency, and over-heparinization is not explained by current knowledge or theories of coagulation. Presumably, blood could be extravasated under these conditions because (1) blood is less viscous and its surface tension altered, or (2) the continuity of the walls of the small vessels depends upon some continuing contribution from blood which is destroyed when the clotting process is delayed or inhibited.

Measurements of surface tension and viscosity have shown no significant changes in hemophilia, prothrombin deficiency, or excessive heparinization. Transfusion of normal dogs, until hematocrit readings of 65 to 70 per cent were obtained, resulted in a considerable increase in viscosity and a decrease in surface tension, but these animals bled just as readily from prothrombin deficiency or over-heparinization as did untransfused controls. The irradiated dog (450 r.) given sufficient blood daily to maintain a hematocrit reading of 70 per cent had similar changes in viscosity and surface tension and developed increased clotting time, thrombocytopenia, and hemorrhage. Anemia and hypoproteinemia produced by the daily withdrawal of blood in normal dogs reduced viscosity and increased surface tension but did not cause these animals to bleed or make them unduly susceptible to hemorrhage from Dicumarol or heparin.

Data are presented which suggest that coagulation is a dynamic and protective process. The activity of prothrombin rapidly falls and may disappear entirely fourteen to twenty hours after hepatectomy. Fibrinogen concentrations decline in a similar manner, though less rapidly. That the removal of the liver is not necessary for the rapid decline of prothrombin is indicated by the rapid disappearance of transfused prothrombin in whole blood or fresh plasma. Apparently the turnover of prothrombin is rapid and nearly complete in twenty-four hours, and that of fibrinogen is only slightly longer (two to three days). The fate of these proteins is not known. It is possible that fibrin is constantly formed and is concerned with the maintenance of the integrity of the vascular wall, and that its formation consumes prothrombin.

2. SIMULTANEOUS CESAREAN SECTION AND SPLENECTOMY IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

W. R. Arrowsmith, M.D., Curtis Tyrone, M.D. (By Invitation), and Champ Lyons, M.D. (By Invitation)
New Orleans, La.

Although splenectomy has been done many times on pregnant patients, we are unable to find any record of simultaneous splenectomy and cesarean section in the management of a complicated pregnancy.

The patient, a 27-year-old white woman, with two preceding spontaneous abortions, was first seen by us in the seventh month of pregnancy, because of purpura. She had a long past history of abnormal bleeding for which no cause had been found. Physical examination showed innumerable petechiae over the legs, scattered purpuric lesions of the rest of the body, and findings compatible with a seven-month pregnancy. Laboratory studies showed an infinitely prolonged bleeding time, platelet count of 2,000 per cubic millimeter, and increased megakaryocytes in the bone marrow. Roentgenogram showed a mild cephalopelvic disproportion.

Because of the significant fetal mortality reported following splenectomy in the third trimester, and because of the relatively minor bleeding, it was decided that the safest course in this patient would be to allow her to try to deliver spontaneously and then perform a splenectomy post partum, early if necessary or later if bleeding was not too excessive. After six hours in labor at term it became apparent that cesarean section would be necessary. A low-flap cesarean section was performed, followed immediately by splenectomy. When the patient entered the operating room the bleeding time was twenty-one minutes; while the abdomen was being closed it was two minutes and thirty seconds. The postoperative course was uneventful and the patient has had no abnormal bleeding for the ensuing thirteen months.

The baby's platelet count at delivery was about 100,000 per cubic millimeter with normal bleeding and clotting times. She is healthy at the age of 13 months, has a normal blood count, and has never had any hemorrhagic phenomena.

The management of this case is not presented as standard treatment for purpura during pregnancy. Rather, it is a hitherto unreported example of the effectiveness of splenectomy in preventing major hemorrhage in a patient with thrombocytopenic purpura in whom another major surgical procedure was necessary as an emergency.

3. THE DETECTION OF INTRACRANIAL TUMORS BY THE USE OF DI-IODO^{131}-FLUORESCEIN

Moses Ashkenazy, M.D. (By Invitation), George V. LeRoy, M.D., Theodore Fields, B.S. (By Invitation), and Loyal Davis, M.D. (By Invitation), Chicago, Ill.

Neoplastic tissues have the ability to combine with fluorescein dye. This combination is temporary, lasting for a period of two to four hours after intravenous injection of the dye. When di-iodo^{131}-fluorescein (radiofluorescein) in amounts of 0.7 to 1.0 mc. is used, the greater radioactivity of the tumor tissue permits one to detect its location by the use of suitably arranged Geiger-Müller counters. This technique has been applied to the diagnostic study of patients with space-filling intracranial lesions. When the lesion is a neoplasm, good

concentration of the radiofluorescein occurs, and the location of the tumor can be established. When the lesion is a nonneoplastic cyst, or hematoma, there is no significant local accumulation of the radioactive material. A description is given of the differential counter method and of the apparatus developed in the Radioisotope Unit, Hines Hospital. The results of the examination of sixty patients with this technique are discussed. In every instance but one, to date, the diagnosis based on the isotope studies has been verified at operation.

4. THE INHIBITION BY NORMAL SYMPATHETIC VASOCONSTRICTOR TONE OF THE SPONTANEOUS DEVELOPMENT OF A COLLATERAL CIRCULATION IN CHRONIC OBLITERATING ARTERIAL DISEASE OF THE LEG

LAWRENCE N. ATLAS, M.D., CLEVELAND, OHIO

In 1937 observations were made which suggested that a collateral circulation would spontaneously develop as a normal physiologic response to chronic ischemia of the leg and foot were it not for the fact that the flow of sympathetic vasoconstrictor nervous impulses to the arteries of the leg and foot acted as an inhibiting influence. To test the validity of this premise a series of cases of chronic thromboangiitis obliterans and arteriosclerosis obliterans of the lower extremities were subjected to lumbar sympathetic ganglionectomy. During the past eleven years a total of 128 operations has been performed on 112 individuals. On the basis of history, age, and physical findings, 32 probably had thromboangiitis obliterans and 80 arteriosclerosis obliterans. The latter group included 20 diabetic subjects.

The original thesis proved to be valid. A gradually progressive, measurable increase in the magnitude of arterial pulsation in the denervated leg has been a constant postoperative finding. The maximum postoperative increase in pulsation frequently was not attained until a year had elapsed and has been maintained, in some instances, for as long as ten years.

This presentation includes a brief discussion of the physiology of collateral circulatory development in the presence of chronic ischemia, the clinical observations giving rise to the original premise, the clinical selection of cases, and the presentation and interpretation of recorded oscillometric evidence of the spontaneous development and prolonged maintenance of an effective collateral circulation in cases of chronic obliterating arterial disease of sympathectomized legs.

5. OCCURRENCE OF ANTIHEMAGGLUTININS AGAINST NEWCASTLE VIRUS IN HUMAN RESPIRATORY INFECTIONS WITH A POSSIBLE INSTANCE OF VIRUS ISOLATION

MALCOLM B. BAWELL, M.D. (BY INVITATION), MARGARET LEGIER, M.S. (BY INVITATION), FRANCES MURREY, B.S. (BY INVITATION), WILLIAM SCHOFIELD, D.V.M. (BY INVITATION), AND G. O. BROUN, M.D., ST. LOUIS, MO.

Newcastle disease, a pneumoencephalitis of fowl, causes human conjunctivitis, and serologic evidence suggests it may cause infections resembling poliomyelitis or influenza. The causative virus agglutinates chicken cells and antihemagglutinins occur in sera of inoculated or infected fowl.

Having isolated this virus from infected chickens, we sought evidence of human infection by serologic studies of patients suffering from influenza or virus pneumonia. Sera of thirty-six such patients collected in the autumn

and winter of 1946-1947 showed antihemagglutinins against Newcastle virus in two instances (5.5 per cent). Examination of sera from thirty-one cases of respiratory infection occurring in the autumn and winter of 1947-1948 revealed antihemagglutinins in ten (32.2 per cent). Many true influenzal infections showed no antihemagglutinins against Newcastle virus. The titer of Newcastle antihemagglutinins was less than 1/100 in all but two instances. All but two of the same sera showed a higher titer of antihemagglutinins against some strain of influenza virus.

Specimens of serum were collected in September, 1948, from 117 healthy medical students, largely Freshmen, who had come to St. Louis from various parts of the country. Of these specimens only one showed the presence of antihemagglutinins against Newcastle virus.

From the excised lung tissue of a case of virus pneumonia which had been followed by chronic atelectasis of the right middle lobe, we apparently isolated a strain of Newcastle virus. The first series of embryonated eggs inoculated with the tissue showed hemagglutinins in the chorioallantoic fluid in the fourth and subsequent transfers. Chickens inoculated with this material developed symptoms similar to Newcastle disease. Virus was recovered from them. Known Newcastle antiserum neutralized hemagglutinins from egg fluid and chick tissue transfers. However, a second series of eggs inoculated with the human lung tissue failed to give evidence of the presence of virus. The patient's serum was not collected for testing until two months after the lobectomy. At this time it showed a titer of 1/40 against the strain of virus isolated from the patient's own lung tissue. It showed a much higher titer against a strain of influenza virus.

The finding of antibodies against Newcastle virus may mean an exposure to the virus or a nonspecific production of antibodies as a result of reactions to influenza and perhaps other viruses. Our findings do not exclude either possibility.

6. PRELIMINARY NOTE ON THE EFFECT OF VITAMIN B_{12} ON THE PAINFUL ASPECTS OF NUTRITIONAL NEUROPATHY

WILLIAM B. BEAN, M.D., MURRAY FRANKLIN, M.D. (BY INVITATION), AND ADOLPH L. SAHS, M.D. (BY INVITATION), IOWA CITY, IOWA

The complexity of the relationship of specific vitamins or food factors to neuropathies which occur in nutritional deficiency states has increased with the discovery of new vitamins, particularly fractions of the B complex. Formerly it was believed that the neuropathy of beriberi was the result of thiamine deprivation. This has been questioned repeatedly without any clear light being thrown on the specific compound or compounds whose relative or absolute deficiency gives rise to neuropathies. Furthermore, it is possible that nonspecific toxins or specific antivitamin or antienzyme factors may be concerned.

Because of this uncertainty and because vitamin B_{12} had succeeded in controlling manifestations of combined system disease in pernicious anemia, we tried vitamin B_{12} in three patients with painful neurological disturbances which followed nutritional deficiency or chronic alcoholism and in eleven persons with painful neurological disorders not apparently related to nutritional factors. Two of the three patients were observed on a control diet poor in B complex vitamin factors for a week prior to administration of vitamin B_{12} intramuscularly in a dose of 15 gamma, after injections of normal salt solution failed to produce any improvement. The third patient, an alcoholic

addict with diabetes, had required narcotics for relief of pain. In these three instances, following the intramuscular administration of 15 gamma of B_{12}, very dramatic relief of the pain occurred within thirty to sixty minutes. In the diabetic patient no further narcotics were required for pain during the hospital stay of two weeks. In the others, pain gradually reappeared within three to five days and responded again but less dramatically to a second injection of vitamin B_{12}. They were then taken off the control diet and fed a nutritious diet high in calories, protein, and vitamins and they continued to show improvement. Eleven patients with brachial neuritis (two), multiple sclerosis (two), diabetic neuritis (two), tabes (one), trigeminal neuralgia (one), monocytic leucemia (one), ruptured disc (one), and alcoholic psychosis (one) were given similar injections of sterile saline and vitamin B_{12}. In no instance was improvement induced, indicating absence of a sedative or analgesic effect for B_{12}. Since nutritional neuropathies are relatively uncommon in this hospital, it has not been possible to make more extensive observations. This preliminary report is presented that others may try out this compound in nutritional neuropathies.

7. EFFECT OF COLD APPLICATION IN PATIENTS WITH ANGINA PECTORIS

BERNARD BERMAN, M.D., CINCINNATI, OHIO

(INTRODUCED BY JOHNSON MCGUIRE, M.D.)

Cold was applied to various body sites in patients with coronary arteriosclerosis and angina of effort. The electrocardiographic changes and general body response were studied.

One refrigerator ice cube, held in contact with the patient's skin, was used in twenty patients with coronary arteriosclerosis and angina of effort. The ice cube was applied to the following sites in different patients: anterior forearm (ulnar surface at the wrist), sides of the nose, chest (region of the right nipple), and the upper abdomen. In order to avoid local chilling of the heart, the precordial region was not used as a site for the test. While applying the ice cube to the nose, the external nares were allowed to remain patent in some patients and occluded in others, in order to determine the effect of chilling the inspired air.

Three patients with angina of effort showed a 1 mm. depression of the S-T segment in one or more of the limb leads following application of cold. One patient showed depression of the S-T segment in Lead I following application of cold to the right chest, although no pain was experienced at this time. Substernal pain followed application of cold to the nose for two minutes. There was prominent depression of the S-T segment in Leads I, II, III at this time. One patient experienced chest pain following application of cold to the nose and wrist for ten minutes, and the pain subsided in approximately three minutes. The electrocardiographic tracing in the limb leads during the bout of pain showed no significant change. One patient developed trigeminal rhythm following application of the ice cube to the nose for five minutes which persisted approximately seven minutes.

Two patients experienced chest pain during the test and two had a delayed reaction of chest pain. One patient, who showed a negative test electrocardiographically following application of cold, had onset of precordial pain approximately five to seven minutes later. The delayed reaction of chest pain was present in another patient, who reported chest pain upon walking from the laboratory. He previously had suffered no episodes of angina for several months prior to the test.

There were no marked changes in blood pressure response noted in any of the patients studied.

This and other preliminary data indicate that a cold stimulus, particularly following a meal, materially increases the positive tests for so-called "ischemia of heart muscle." This may be of clinical value in detecting coronary insufficiency.

It would appear that the cold stimulation of the external skin of the face, especially about the nose, is one of the factors which may contribute toward initiating attacks of angina in patients walking against a cold wind.

8. EVALUATION OF THE ZINC SULFATE TURBIDITY AND TOTAL LIPID DETERMINATIONS IN LIVER DISEASE

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(WITH THE TECHNICAL ASSISTANCE OF LILA DOHM, B.S., AND ELIZABETH ROBINSON, B.S.)

In order to assess the clinical value of the zinc sulfate turbidity and total lipid determinations as described by Kunkel, a series of 871 consecutive "liver profiles" were studied. These new turbidity tests were contrasted with the routine liver "function" tests simultaneously performed. The 871 determinations were made in approximately 400 patients, more than half of whom had clinical evidence suggestive of liver disease. Needle biopsy of the liver was performed in many instances.

The zinc sulfate turbidity test was contrasted with the thymol turbidity test. 12.0 units was chosen as the upper limit of normal for the zinc sulfate turbidity, and 5.0 units for the thymol turbidity. The cases were divided into the following groups: cirrhosis (nutritional, postnecrotic, and portal), hepatitis (infectious and homologous serum), obstructive jaundice, carcinoma (primary and secondary), and miscellaneous. The miscellaneous group included patients with nonhepatic disease as well as a few with granuloma of the liver and fatty infiltration of the liver.

The zinc sulfate turbidity was elevated while the thymol turbidity was normal in 19.4 per cent of the determinations. The thymol turbidity was elevated and the zinc sulfate turbidity normal in 5.6 per cent of the determinations.

When the determinations were divided according to diagnosis, the following significant data appeared.

(a) In cirrhosis: the zinc sulfate turbidity was elevated and the thymol turbidity was normal in 16.8 per cent of the tests; whereas the zinc sulfate turbidity was normal and the thymol turbidity elevated in 3.7 per cent. (b) In hepatitis: the zinc sulfate was elevated and the thymol turbidity was normal in 8.5 per cent of the tests; whereas the zinc sulfate turbidity was normal and the thymol turbidity elevated in only 0.7 per cent. (c) In obstructive jaundice and carcinoma of the liver: the zinc sulfate turbidity and thymol turbidity tests did not show the significant differences seen in cirrhosis and hepatitis.

It was felt that the zinc sulfate turbidity test was particularly useful in cases of cirrhosis of the liver.

The values obtained for total lipids using the method of Kunkel were contrasted with the values obtained for cholesterol using the method of Bloor. A total of 167 determinations were so compared. It was seen that the values of

the total lipid and cholesterol roughly paralleled one another. The ratio of total lipid to cholesterol was determined and it was found to be 3.76 ± 0.7. In 17.9 per cent of the determinations this ratio was exceeded. Most of these were instances of hepatitis and/or cirrhosis. The significance of these ratios is to be discussed.

It was felt that these new turbidity tests provide useful information and deserve inclusion in routine liver function studies.

9. AN ANTIDIURETIC SUBSTANCE IN THE URINE OF PATIENTS WITH CARDIAC FAILURE

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Recently, much doubt has been cast upon the conventional explanations of the mechanisms involved during cardiac decompensation. Because of the controversial state of this subject at present, it was felt that examination of other factors involved in water balance would be worth while. Therefore, patients with congestive heart failure were studied for the presence of increased amounts of antidiuretic material excreted in the urine.

Patients with definite heart failure were selected for study. None with associated kidney disease or cirrhosis were used. Urines from normal subjects and patients with mental diseases were used as controls. Urines were acidified to a pH of 5.5 to 6.5 with 3 per cent acetic acid, evaporated at room temperature beneath a fan, and then dialyzed in a cellophane sac for six to twenty-four hours. Urines were concentrated so that a fifteen-minute output sample would equal 2 to 4 c.c., and this amount was used as the test dose. Assay was performed on trained normal female dogs in which water diuresis had been produced by giving 35 c.c. per kilogram body weight by stomach tube. Urine samples were collected through an indwelling catheter. A decrease in urine output following intravenous injection of the test material was used as a measure of antidiuretic activity and this was compared with the change in urine output following the injection of known amounts of Pitressin.

Ten patients were studied. Of these, three had hypertensive cardiovascular disease, three had arteriosclerotic heart disease, three had rheumatic heart disease, and two had cor pulmonale. Nine of these were found to have amounts of antidiuretic substance equivalent to 0.5 to 1.0 mμ of Pitressin in a fifteen-minute urine sample. None of the controls showed any antidiuretic activity in their urine samples. Investigations now in progress indicate that this antidiuretic material is not the same as commercially prepared Pitressin.

10. VICARIOUS EXCRETION BY MEANS OF PERGASTRIC INTESTINAL PERFUSION

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Pergastric intestinal perfusion was carried out in nine preterminal uremic patients as a means of vicarious excretion. The subjects were placed on a Bradford frame with head-end elevated. The perfusing fluid was introduced into the stomach and collected from a rectal tube after transit through the entire intestine. To prevent overdistention of the stomach and vomiting, suction was

applied at the cardia through a second lumen of the gastric tube. Such a scheme avoided many of the disadvantages of other artificial means of vicarious nitrogenous excretion. It required no elaborate equipment, no surgical procedure, no preparation of sterile solutions, and avoided the danger of thrombosis or peritonitis. It could be instituted with a minimum of delay.

A significant fall in the serum nonprotein nitrogen concentration was recognizable after six to eight hours of perfusion. The nonprotein nitrogen level of the intestinal perfusate was directly proportional to the serum nonprotein nitrogen level and inversely to the rate of flow. The mean intestinal nitrogenous excretion was equivalent to a urea clearance of 13.6 c.c. per minute (range, 6.9 to 21.0 c.c.). At the optimal rate of flow of 2 liters per hour, the clearance was of the order of 21.0 c.c. per minute. In contrast, gastric perfusion alone was only about one-sixth as effective.

Blood levels of creatinine, phosphate, and phenols were not appreciably lowered. Alteration of serum sodium, chloride, and bicarbonate levels generally was toward normal.

Serum potassium levels dropped considerably in all instances, though less so when the potassium concentration of the perfusate was made 40 mg. per 100 cubic centimeters. In prolonged perfusions, the serum potassium might fall to the dangerously low level of 12 mg. per 100 cubic centimeters. The high concentration of potassium in the intestinal perfusate implied the nonosmotic secretion of potassium in the intestinal juice. The total negative balance of potassium was as much as five times as great as the total extracellular content of potassium. The lost potassium was therefore largely intracellular. This made the extent of the fall in serum potassium concentration unpredictable. In perfusions of more than six to eight hours, the potassium loss might be dangerous. This is the limiting factor in the use of this method, which otherwise appeared to be of distinct potential value in prolonging life.

11. EXPERIENCE WITH HEPARIN-PROTAMINE TITRATION

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Allen and co-workers first noted that in certain hemorrhagic states a heparinemia-like condition exists which will respond to the parenteral administration of protamine or toluidine blue. They devised a heparin-protamine titration technique to detect this state. Using a simplification of this titration, suitable for clinical laboratory analysis, ninety-one tests were performed using forty-eight patients with various hematologic diseases and twenty-three normal subjects. One cubic centimeter lots of blood rendered incoagulable with 90 gamma of heparin are titrated against selected 0.02 c.c. increments of a standard protamine solution until an end point of coagulation at room temperature in one hour is achieved. Normal end points range from 0.12 to 0.18 c.c. of solution, depending on potency of successive batches. Tests were read as −, 0, +, ++, or +++ according to the relationship in 0.02 c.c. increments of the patient to the control value. Of twenty-three tests run on normal subjects, three were −, eleven were 0, and nine were ±. Thus a ± value is of no significance. Six patients with thrombocytopenic purpura, four with acute leucemia, seven with chronic leucemia, two with aplastic anemia, nine with pernicious anemia, four with polycythemia, three with miscellaneous anemias, five with prolonged roentgen therapy, six with obscure bleeding problems, and one each with multiple myeloma, Hodgkin's disease, and myeloid metaplasia of the spleen were tested. Of these, only three of the four acute leucemias and two of the seven

chronic leucemias showed a significant increase. Interestingly, three of the six obscure bleeding problems showed a − reaction, possibly indicating a compensatory increased tolerance for heparin.

12. DISTRIBUTION OF BLOOD TYPES IN THE LEUCEMIAS

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Of five hundred thirty-two patients with leucemia studied, most of whom have been observed over the past twelve years, Landsteiner blood groups were recorded in one hundred thirty-seven and Rh types in thirty. Four of those with known Landsteiner types were Negro; all the others were white. Of those typed, 42 per cent suffered from acute leucemia, 45 per cent from chronic, 5 per cent from monocytic, and 7 per cent from other types of leucemia. The larger group studied consisted of 31 per cent acute, 52 per cent chronic, 6 per cent monocytic, and 11 per cent miscellaneous varieties. Thus blood groups were recorded in a significantly greater percentage of acute conditions than chronic. The distribution of blood types among the one hundred thirty-seven patients was: O, 39 per cent; A, 41 per cent; B, 13 per cent; and AB, 7 per cent. The distribution of blood types as recorded in over four thousand, five hundred recent, routine blood bank tests is: O, 44.1 per cent; A, 38.6 per cent; B, 13.2 per cent; and AB, 4.2 per cent. If the figures from the cases of leucemia are compared by chi-square with those of the larger hospital group, the probability of differences being due solely to random selection is 0.55. Thus, there is no significant difference of distribution between the two groups. Similarly there is no significant variability of blood type distribution in the smaller groups of chronic myelogenous leucemia, chronic lymphatic leucemia, and the pooled cases of acute leucemia.

Of the thirty patients Rh typed, 77 per cent were positive, 23 per cent negative. Comparison by chi-square with the published figures of 85 per cent positive and 15 per cent negative gives a probability of 0.20, revealing no significant difference between the leucemic and the general groups.

13. OCCURRENCE OF HEMOPHILIA IN FEMALES

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Hemophilia is considered ordinarily as a disease of the male sex only, inherited as a sex-linked recessive characteristic through the x-chromosome. Of the possible types of crosses in human hemophilia, only two, conductor female × normal male (Hh × HY) and normal female × hemophilic male (HH × hY), are well established. Rare instances are reported of matings between conductor females and hemophilic males. In this cross, Hh × hY, half of the female offspring should be of the genotype hh and would be expected to be bleeders. However, authentic cases of female hemophilia have not been described. As a result it has been postulated that the genic combination hh is lethal, or that the bleeding tendency does not become manifest in females.

Recently a strain of dogs with an inherited bleeding disease was discovered. On the basis of the Hh × HY cross, a sex-linked type of inheritance was demonstrated. The clotting defect appears to be identical to that in human hemophilia. As a result of a breeding program carried on in our laboratory, bleeder males have been reared to maturity. This report deals

with the mating of bleeder males with conductor females. Six litters from such matings have been studied. Nearly half of the males were hemophiliac, while the remainder were normal. Approximately half of the females were hemophiliac, while the rest showed no evidence of the disease. The distribution into bleeders and nonbleeders in these litters was close to the expected ratio of 1:1 for the cross, Hh × hY.

The clotting defect in the affected animals was the same, regardless of sex. All showed consistently prolonged clotting times, normal bleeding times, normal prothrombin values, and a slow disappearance of prothrombin from clotting blood in spite of normal platelet values. The clotting defect was corrected in vitro by normal plasma or small amounts of thromboplastin. Further studies indicated that the bleeder females, just like the bleeder males, lack a plasma factor required for platelet utilization.

No sex difference in the bleeding tendency was observed. Hemarthroses and subcutaneous hematomas have occurred frequently. Some animals have had evidence of internal bleeding. Hemorrhagic episodes have been controlled by plasma transfusions.

The occurrence of hemophilia in female dogs suggests that the genic combination hh in human beings is not lethal. Absence of human cases may be due to lack of opportunity for their occurrence, or to inadequate investigation of possible cases.

14. OBSERVATIONS ON THE EPIDEMIOLOGY OF INFECTIOUS HEPATITIS

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The occurrence of infectious hepatitis was studied in two widely separated areas in Wisconsin. One region, in the north-central section, consists of a city of 10,000 inhabitants and surrounding rural communities. The other, 250 miles away in the southwestern section, is a prosperous farming region. The geology of the areas is different. The chief method of study consisted of interviews with each individual who had had the disease and with his suspicious contacts. Most of the cases were discovered in this way. An attempt was made to ascertain pertinent facts concerning the development of each case and to document the individual and environmental factors. Similar interviews were obtained with 60 families in which hepatitis had not occurred. Sanitary surveys were made by the State Board of Health, and other data were obtained relative to terrain, climatic variations, and disease rates. For the purpose of this study, jaundice was made a mandatory criterion for diagnosis.

In the northern area, 168 cases of infectious hepatitis occurred, beginning in January, 1947. In the south, 31 cases had appeared, the first in December, 1945. The disease appeared in all months of the year, with a significantly greater number in winter. The greatest number of cases occurred in patients between the ages of 10 and 30, the disease rarely appearing in preschool children or persons over 50. There was a clear tendency to distribution by families. In the 115 families concerned, there were 199 cases (1.7 persons per family). Of the 579 persons in these families, 34 per cent developed the recognizable syndrome. Members of other families in the vicinity were not involved, even though all activities were shared, so far as could be determined. The occurrence of the disease seemed to present a pattern by groups. In the southern region it was confined to an area of approximately four square miles.

Various environmental factors were studied. The drinking water from wells was found to be contaminated in 30 per cent of homes where cases occurred and in only 5 per cent of homes which had been free of the disease. Other considerations tend to minimize the importance of this as the source for transmission. The occurrence of the disease in localized areas by groups seemed to be the most important aspect of these observations.

15. RATES OF TURNOVER AND BIOLOGIC DECAY OF CHLORIDE AND CHLORIDE SPACE IN DOGS DETERMINED WITH THE LONG-LIFE ISOTOPE, Cl^{36}

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The long-life radiochloride, Cl^{36} ($T_{½} \simeq 2 \times 10^6$ years), made possible the study of the early concentration-time course in the blood serum, biologic decay rates, and rates of turnover of chloride in six normal dogs observed continuously for eighteen to thirty-one days under controlled metabolic conditions. These experiments yielded the following information.

(1) The mean concentration-time course of Cl^{36} in the serum for the first sixty minutes after intravenous administration can be expressed by the multiple exponential equation

$$CN_t = 1250e^{-2.079t} + 680e^{-0.308t} + 144e^{-0.0377t} + 674e^{-0.000175t},$$

where

CN_t = concentration in the serum at any time t;
e = natural log.

(2) All the seral chloride leaves the serum each minute, and about one-third of the nonseral chloride returns to the serum each minute. Thus in a dog weighing 4.54 kilograms (10 pounds) with a chloride mass of 5.5 Gm., about 2.75 Gm. of chloride pass into and out of the serum each minute.

(3) The mean $C_{½}$ value for the serum was 2.51 days (range, 2.16 to 3.00), the mean $U_{½}$ 3.50 days (range, 2.75 to 4.00), and the mean $E_{½}$ 3.40 days (range, 2.75 to 3.80).

(4) The mean recovery was 91 per cent of that injected (range, 83 to 97)—88 per cent (range, 81 to 90) in the urine and 2.6 per cent (range, 0.6 to 5.9) in the feces.

(5) The mean chloride space in "serum equivalents" was 35 per cent of body weight (range, 32 to 38) and the mean total body chloride 6.84 Gm. for a dog weighing 4.54 kilograms.

(6) These and other data to be presented indicate that: (a) The chloride space is larger than previously reported. (b) Chloride cannot be employed as a measure of extracellular fluid space. (c) Chloride is present in considerable quantities outside the extracellular fluids. (d) Man would require a daily intake of 43 Gm. NaCl or 15 to 22 lb. of food to have a diet comparable to that of a dog weighing 4.54 kilograms. (e) A diet of 200 mg. sodium for a man weighing 70 kilograms is equivalent to a diet of 13 mg. for a dog weighing 4.54 kilograms. Thus, the usual studies of low sodium metabolism in small animals with "hypertension" must be evaluated cautiously.

The importance of these studies in planning tracer experiments with chloride and bromide and in understanding the metabolism and excretion of chloride and bromide, edematous states, intoxication due to radiochloride and radiobromide in atomic warfare, and other physiologic processes is evident from the data.

16. ORAL ADMINISTRATION OF VITAMIN B_{12} IN PERNICIOUS ANEMIA

II. Studies on the Nature and Source of Intrinsic Factor

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Vitamin B_{12}, when administered parenterally to patients having pernicious anemia in relapse, behaves in the same manner as does the active principle in liver. However, when vitamin B_{12} is administered orally it acts as does extrinsic factor, as Berk and co-workers, Hall and associates, and others have shown. Since patients with pernicious anemia lack intrinsic factor, orally administered vitamin B_{12} is not utilized, in most cases at least, unless the intrinsic factor is given also, either simultaneously or within a period of six hours (Castle and associates). If the quantity of vitamin B_{12} is kept constant, various unknowns can be tested for the presence or absence of intrinsic factor. We have utilized this procedure with seventeen patients having pernicious anemia in severe relapse. The following data have been gathered from these studies.

(1) Intrinsic factor is present in fresh, Berkefeld-filtered, pooled human gastric juice. (2) It is also present in extracts of hog stomach and duodenum. (3) It is destroyed by heating to a temperature of 63° C. for thirty minutes. (4) It is not destroyed in human gastric juice when the latter is stored at a pH of 1.7 at a temperature of 5° C. for three months. (5) Trichloracetic acid and cold alcohol (95 per cent) precipitates from human gastric juice (prepared for us by Dr. J. L. Bollman) did not contain the intrinsic factor. (6) In vitro mixtures of pooled human gastric juice and vitamin B_{12}, after standing at room temperatures for twenty-four hours, were heated to 70° C. for sixty minutes. When these were administered orally to a patient with pernicious anemia in relapse, no hematopoietic response was elicited. (7) The minimal amount of human gastric juice required to produce optimal hematopoietic responses when administered daily by mouth with 5 or 10 μg of vitamin B_{12} has not been accurately determined, but in our experience the daily administration of 75 c.c., plus vitamin B_{12}, has given optimal results. (8) Variation in the response to large quantities of vitamin B_{12} administered alone by mouth has been noted. One patient failed to show a hematopoietic response to a single oral dose of 1,000 μg of vitamin B_{12} concentrate. A second patient exhibited a suboptimal response to a single dose of 75 μg of crystalline material. From this, and similar observations by others, it is possible to theorize that lack of intrinsic factor in patients with pernicious anemia is not absolute.

17. CONGESTIVE HEART FAILURE AND HYPONATREMIA: UNTOWARD EFFECTS OF MERCURIAL DIURESIS

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Recent literature concerning the treatment of congestive heart failure has emphasized the value of dietary salt restriction and the frequent use of mercurial diuretics. One of the complications incident to the rapid diuresis produced by this type of therapy is the profound change in the electrolyte balance.

Such an instance was encountered in the case of a 50-year-old man who became critically ill and comatose as the result of treatment of his severe congestive failure with low salt diet and mercurial diuretics. It was not until three

days subsequent to the appearance of coma, when the plasma chloride concentration was found to be low, that the true pathogenesis of his condition was suspected and treatment with sodium chloride solution seemed justified. The patient improved clinically and this improvement paralleled the restoration of electrolyte balance. Since this initial experience eleven other patients presenting a similar therapeutic problem have been treated.

In patients with well-established congestive failure with edema the extracellular fluid compartment is expanded. The removal of the excess fluid in the extracellular space may be accomplished by a combination of low salt intake and mercurial diuretics. During the excretion of this fluid, however, sodium and chloride are removed at a relatively faster rate than water. Consequently, the sodium chloride values fall and the fluid becomes hypotonic. In the presence of subnormal plasma sodium and chloride concentration the diuretic response to organic materials is inhibited. Clinically the situation which at this point presents itself is one of a patient with chronic cardiac failure who in spite of vigorous treatment is getting worse rather than better and does not respond to mercurial diuretics. Dizziness, drowsiness, muscular pains, and apathy appear, and if correction with adequate amounts of sodium chloride is not made in time, confusion, convulsions, and death may result.

18. METABOLIC CHANGES INDUCED BY SUBTOTAL ADRENALECTOMY RESULTING IN CURE OF CUSHING'S SYNDROME; EFFECTS OF LATER ADMINISTRATION OF ACTH

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A metabolic study was carried out upon a 22-year-old woman with typical Cushing's syndrome proved to be due to bilateral adrenocortical hyperplasia. It affords comparative data in the same person under three separate conditions: (1) before subtotal adrenalectomy, (2) after metabolic and physical normaley had been established by removal of more than 90 per cent of adrenal tissue, and (3) during stimulation of the adrenocortical remnant with large amounts of ACTH (100 mg. per day Armour Standard). The data include observations upon the metabolism of nitrogen, carbohydrate, uric acid, glutathione, cholesterol, and electrolytes as well as daily excretions of 17-ketosteroids and of "11-oxysteroids."

To be emphasized are the following results and conclusions.

(1) Before operation, fasting blood glutathione averaged 26 mg. per cent (highest value, 28 mg. per cent) and carbohydrate tolerance was greatly impaired. Intravenous reduced glutathione (GSH) abruptly converted the diabetic tolerance to normal, but it quickly reverted to the diabetic type. Four months after operation fasting blood glutathione averaged 43 mg. per cent (lowest value, 39 mg. per cent). Carbohydrate tolerance was then normal and has remained so.

These observations are in accord with our previous reports upon the role of GSH in ACTH-induced diabetes in normal human beings. A deficiency of free sulfhydryl (–SH) groups appears to be importantly involved in the pathogenesis of the diabetes observed in Cushing's syndrome.

(2) Excretion of 17-KS and of 11-OS (both elevated threefold preoperatively) became persistently normal after adrenalectomy. ACTH acting upon the adrenal remnant returned these values to their preoperative levels! They became normal again upon cessation of ACTH.

Upon sufficient stimulation and for, at least, a short period of time, a small remnant of adrenal cortex can give rise to the same quantity of steroidal end products as had been produced by two hyperplastic glands during the active stage of Cushing's syndrome. Conversely, the hyperplastic adrenals of active Cushing's syndrome are not receiving maximal functional stimulation from endogenous ACTH.

(3) Despite an ACTH-induced return of excretory corticosteroids to preoperative levels, some of the preoperative metabolic aberrations failed to reappear. This was true also of the preoperative vascular hypertension.

It is likely that some of the clinical and metabolic manifestations of increased cortical activity are a function of time as well as of intensity, that mildly increased cortical activity of sufficient duration eventually will manifest itself. This may be important with respect to therapeutic uses of ACTH.

19. A CLINICAL-PATHOLOGICAL SURVEY OF 108 TUBERCULOUS PATIENTS

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Autopsies were performed on 108 patients who had active tuberculosis. The diagnosis of tuberculosis was established by clinical means in 95 (Group A), but the disease was not recognized before death in the other 13 patients (Group B).

Group A.—Tuberculosis was the primary cause of death in 73 of the cases in which it was diagnosed before death, but in 18 patients other diseases than tuberculosis were the cause of death. In 13 of these 18 the nontuberculous disease causing death was recognized clinically; in the other 5 it was not suspected. There were four other patients in this group in whom it appeared that the tuberculous process and another disease were of equal importance in the patient's demise. Adequate attempts were made in the treatment of recognized nontuberculous diseases in this group, but in those five patients who had clinically uncovered diseases early treatment might have helped. There were the following conditions unrecognized before death in the five patients in whom the clinical diagnosis of active tuberculosis was made: carcinoma of the pancreas, heart failure due to arteriosclerotic heart disease, pulmonary infarction, and two cases of pulmonary atelectasis.

Group B.—Analysis of the 13 patients who had active tuberculosis discovered only at the time of the postmortem showed that two died primarily of the tuberculous process, one from acutely disseminated miliary tuberculosis and one from tuberculous peritonitis. The other eleven patients had clinically recognizable nontuberculous diseases and were properly though unsuccessfully treated. The clinically unrecognized tuberculosis involved the lungs and respiratory passages in all but one patient in whom primary tuberculosis of the kidney was found at autopsy. The other diseases which caused death in this group were generally so acute, severe, or advanced that treatment of the tuberculous lesion would have been of little avail.

Accuracy of clinical diagnosis involving both the tuberculosis and other associated diseases in the group of 108 patients was 121 (86 per cent) in 141 instances. Active tuberculosis was missed clinically in 13 (12 per cent) of the total group studied. Nontuberculous diseases went undiagnosed pre mortem in 6.5 per cent (7).

Tuberculosis services need to seek consultation from other branches of medicine and surgery, as much as do medical and surgical services need the help of pulmonary disease students. This need should encourage hospitals to provide frequent cross consultations. Close proximity of tuberculous hospital and sanitorium units to other medical and surgical units will aid in this endeavor. Therapeutic advances not only in tuberculosis but in other diseases as well will then be readily available to the patients.

20. POLYCYTHEMIA VERA WITH HEPATIC VEIN THROMBOSIS: CASE REPORT WITH SERIAL LIVER BIOPSIES AND APPARENT RECOVERY

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A case is presented of a patient who apparently recovered from spontaneous thrombosis of the hepatic vein (Chiari's syndrome). It is believed to be the first case to be reported in which the patient recovered.

Case Report.—A 39-year-old shipyard machinist came to the Ochsner Clinic on Aug. 30, 1948, because of weakness, failure to gain weight, and a feeling of fullness in the abdomen of ten months' duration. Three weeks before admission, edema of the legs and ankles and slowly progressive enlargement of the abdomen were noted.

Physical examination and laboratory studies revealed the combined features of polycythemia vera and severe hepatic disease. The patient had a florid complexion with dilatation of superficial vessels and engorgement of the retinal veins. Spider angiomas were noted over the shoulders and chest. The abdomen was filled with fluid. The edge of the liver could be felt about 10 cm. below the right costal margin and there was considerable enlargement of the left lobe. The spleen was ballotable 6 cm. below the left costal margin. Pitting edema was apparent in both ankles and pretibial areas.

There was pronounced elevation of the red blood cell count, hemoglobin, hematocrit, white blood cell count, and platelets. The serum bilirubin value was elevated to a total of 3 mg. per cent; bromsulfalein test showed 46 per cent retention in forty-five minutes; urine urobilinogen excretion was increased, and prothrombin time was 27.8 per cent of normal.

The patient was hospitalized on September 6 for paracentesis and repeat phlebotomies, and 7 mc. of radioactive phosphorus were given intravenously. Initial liver biopsy revealed considerable engorgement of the sinusoids in the central area of the liver with pressure atrophy and beginning fibrous replacement. A small branch of the hepatic vein was seen in one section with a recanalizing thrombus occupying the greater part of its lumen. Despite repeated low prothrombin times, Dicumarol therapy was instituted. Because of recurrent ascites and hemorrhage with paracentesis a peritoneal button was inserted on October 23. With institution of a low sodium diet and repeated administration of mercurial diuretics the patient improved steadily. The liver and spleen showed progressive decrease in size, and on discharge, Dec. 2, 1948, the patient was free of edema and bromsulfalein retention was reduced to 13 per cent in forty-five minutes and total serum bilirubin to 1.16 mg. per cent.

During the next few months the patient required hospitalization once for slight bleeding due to Dicumarol poisoning and once for an intercurrent acute infection. Since that time he has continued to improve, has gained weight,

and is now working full time without difficulty. Two subsequent liver biopsies have shown progressive improvement. P^{32} has been given once since discharge.

The only usual sign of hepatic vein thrombosis which this patient did not exhibit was the presence of pain in the right upper abdominal quadrant. His recovery is believed to be related to the establishment of unequivocal diagnosis by means of liver biopsy and prompt institution of therapy for the polycythemia, with additional control of the ascites and clotting tendencies of the blood during the period of readjustment.

21. RENIN SENSITIVITY AND HYPERTENSINOGEN LEVELS IN ADRENALECTOMIZED DOGS

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Studies undertaken in an attempt to clarify the relationship of the adrenal gland to renin sensitivity and hypertensinogen levels revealed a definite vascular responsiveness to renin and the presence of hypertensinogen in adrenalectomized dogs in both the immediate postoperative period and after frank adrenal insufficiency had developed.

In four animals, after determination of blood pressure responses to 1 unit of angiotonin and 1 mg. of renin, bilateral adrenalectomy was done in two stages. Four days following the second stage, when definite hypotension and pronounced increase in the blood urea nitrogen indicated frank adrenal insufficiency, the renin and angiotonin responses were determined again in the anesthetized animals. Hypertensinogen assays were made on control and experimental animals in these instances. In three of the dogs the intravenous injection of 1 mg. produced essentially the same rise after as before adrenalectomy; whereas in one dog the response was somewhat less but was proportionately as great when calculated as a percentage of the basal blood pressure. In the hypertensinogen assays the amount of this substance was normal in two and questionably reduced in a third.

In four other dogs anesthetized with pentobarbital sodium four days following the second stage of adrenalectomy, when advanced adrenal insufficiency had developed, injection of 1 mg. of renin produced a rise in blood pressure in three instances equal to that produced by 1 to 2 units of angiotonin and in the fourth a smaller rise. In one of these animals 50 mg. of desoxycorticosterone glucoside did not increase the vascular response to injected renin. In two dogs in which the second stage adrenalectomy was done immediately prior to assay there was no essential change in the renin response before or after second stage adrenalectomy. In these dogs tachyphylaxis to renin was as difficult to produce after adrenalectomy as it was before.

In one dog studied immediately after bilateral adrenalectomy, the blood pressure subsequently dropped to 10 mm. of mercury and little renin response could be elicited whereas the angiotonin response was somewhat higher though still considerably reduced. In one normal dog, rendered unresponsive to renin by repeated injections, 50 mg. of desoxycorticosterone glucoside did not materially hasten recovery.

22. FURTHER STUDIES ON EFFECT OF DESOXYCORTICOSTERONE ACETATE IN EXPERIMENTAL HYPERTENSION

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Studies of the blood pressure response in hypertensive and normal dogs revealed: (1) a usual but not invariable transient pressor effect from intravenous injection of 2 c.c. of propylene glycol; (2) no further effect from the addition of 5 mg. of desoxycorticosterone to this material; (3) abolition of this response by a local or general anesthetic, and (4) from continuous subcutaneous administration of desoxycorticosteorne acetate in sesame oil, a sustained and equal pressor effect which could be abolished by elimination of sodium from the diet and quickly precipitated by addition of large amounts of sodium during administration.

In all instances direct blood pressures were taken by femoral arterial puncture and the use of a mercury manometer. Hypertension was induced in the dogs either by use of perirenal silk wrapping or by application of Goldblatt clamps. Several months were allowed to elapse for standardization of pre-experimental pressures.

In thirty-eight determinations on five hypertensive and thirteen normal unanesthetized dogs, no difference in response to the injection of 2 c.c. of propylene glycol alone and 2 c.c. of propylene glycol with 5 mg. of desoxycorticosterone acetate was noted. In most instances a transient 30 mm. rise in pressure was produced by the injection of both desoxycorticosterone and propylene glycol, and propylene glycol alone. This was believed to be the result of phlebalgia, since it was abolished by anesthesia in the dogs.

In experiments conducted over a long period, four hypertensive and two control dogs were given daily injections of desoxycorticosterone acetate in sesame oil in doses of 0.15 to 1.28 mg. per kilogram of body weight. In nine of ten experimental peroids of six weeks each, sustained blood pressure rises of 25 to 50 mm. of mercury were noted. These were sustained at first but gradually tended to return toward normal, and in repeated periods in the same dog tended to be of shorter duration and less magnitude. In such instances, however, increasing the dose of desoxycorticosterone could again augment blood pressure response. These responses, which were of similar magnitude in hypertensive and control animals, were preventable by the use of low sodium intake and precipitable by the addition of sodium chloride to the diet in both hypertensive and control animals. In one of four trial periods in hypertensive animals forced feedings of sodium chloride alone produced a similar rise in arterial pressure.

23. AN EFFECTIVE METHOD FOR THE PREVENTION OF RHEUMATIC FEVER AFTER THE DEVELOPMENT OF A STREPTOCOCCAL INFECTION

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From January through June, 1949, at Fort Francis E. Warren, over 1,650 consecutive cases of exudative pharyngitis and tonsillitis were observed. These cases were divided into control and treated groups of approximately the same size according to Air Force serial number. Treatment consisted of the parenteral administration of crystalline procaine penicillin G (suspended in peanut oil containing 2 per cent aluminum monostearate) in doses of 300,000 units on admission and again in seventy-two hours. Later in the study the dosage schedule was changed to 300,000 units on admission, 300,000 units at forty-eight hours, and 600,000 units at ninety-six hours. Studies for the detection of rheumatic fever were performed between the third and fourth week following the initial infection. Throat cultures and blood specimens were obtained from all patients on admission and at the time of re-examination.

That these infections were almost exclusively streptococcal in origin was established by the presence of group A streptococci in approximately 80 per cent of all the original throat cultures and a diagnostic increase of the antistreptolysin "O" titer in the convalescent blood of about 80 per cent of the control group.

In the entire group, twenty-seven patients developed symptoms suggestive of acute rheumatic fever during a period of from ten to thirty-five days following the initial streptococcal infection. Of these, a definite diagnosis of acute rheumatic fever was made in nineteen patients, seventeen of whom were in the control group and two in the treated group. A diagnosis of possible acute rheumatic fever was made in eight patients, six of whom were in the control group and two in the treated group.

It is concluded that the adequate early treatment with penicillin of streptococcal infections will prevent rheumatic fever in the majority of cases.

24. A COMPARISON OF TUBERCULIN AND ARTHUS TYPES OF HYPERSENSITIVITY; IN VIVO OBSERVATION IN THE RABBIT EAR CHAMBER

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(Introduced by Robert G. Bloch, M.D.)

Using the rabbit ear chamber technique it has been possible to compare in vivo the difference between tuberculin and Arthus types of hypersensitivity. The rabbit ear chamber provides a thin layer of living vascularized tissue 40 to 50 μ thick which can be inoculated directly. Microscopic observations can be made at frequent intervals under the highest magnification, and the dynamics of tissue change can be watched and recorded with Kodachrome motion pictures.

The tuberculin reaction was studied in five chambers inoculated directly with old tuberculin. One animal was sensitized with bovine tubercle bacilli, strain Ravenel RV, and the others, with BCG. Dilatation of blood vessels occurred during the first thirty minutes after inoculation and persisted for at least seventy-two hours. Generalized sticking of leucocytes to vascular endothelium developed during the first thirty minutes, persisted for forty-eight to seventy-two hours, but decreased in extent after thirty-six hours. Diapedesis of white blood cells resulted in the accumulation of considerable generalized exudate within two to five hours after inoculation. The exudate increased in density during the first thirty-six hours. In three experiments localized hemoconcentration due to seepage of plasma through damaged endothelium was followed by stasis and complete thrombosis of small venules. This occurred in the regions of densest exudate twenty-two to thirty-six hours after inoculation.

The Arthus reaction was studied in six chambers using both bovine albumin fraction V and horse serum as antigens. Immediately following direct inoculation of antigen into the chamber in the sensitized animal, platelet thrombi formed in venules and washed away in the general circulation. Within fifteen to thirty minutes there was considerable admixture of white blood cells with platelets in the thrombi which continued to form. Twenty to sixty minutes after inoculation sticking of large clumps of white blood cells and platelets to vascular endothelium was observed in many vessels. Simultaneously other vessels thrombosed completely and plugs of platelets and white blood cells could be seen separating areas of densely packed red blood cells. Dense exudate developed in the regions where sticking was marked, but little or no exudate accumulated around vessels which thrombosed early. The peak of the reaction occurred after eight to ten hours, although sticking of white blood cells, vascular dilatation, and infrequent formation of new platelet thrombi could be observed after twenty-four hours.

Endothelial damage characterized both types of hypersensitivity. In the tuberculin type, damage was either primary or initiated by local tissue damage. In the Arthus type it seemed to be initiated by an intravascular reaction.

25. ELECTROCARDIOGRAPHIC PATTERNS IN PERSONS OVER 80

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Unipolar multiple chest and limb lead electrocardiograms, in addition to the standard leads, were obtained upon one hundred patients over 80 years of age at the Oak Forest Infirmary and the Illinois Masonic Hospital. The abnormalities are tabulated, the largest groups being: first degree heart block, twenty-nine cases; left ventricular hypertrophy, twenty-nine cases; bundle branch block, thirteen cases; myocardial infarction, eleven cases. The establishment of the cardiac position using the unipolar leads explained the axis deviation found in the standard leads: in fifty-three cases of left axis deviation the heart was in the horizontal or semihorizontal position.

Twenty-one electrocardiograms were considered normal using the criteria established by Myers and associates. Of these only fifteen were normal to careful physical examination. Of forty-five patients considered normal to physical examination, thirty cases showed abnormal electrocardiograms. Arteriosclerosis and varying degrees of heart failure accounted in the majority for the physical abnormalities encountered.

Our patients being older than the patients of most other published series, together with the use of multiple chest leads (V_4R, V_3R, V_{1-8}), as well as the unipolar limb leads, makes comparison with earlier series difficult. The advantages of the additional leads to electrocardiographic interpretation again become apparent.

26. CARDIODYNAMIC AND RENAL STUDIES IN CHRONIC PERICARDITIS WITH EFFUSION, WITH PARTICULAR REFERENCE TO THE MECHANISMS OF FLUID ACCUMULATION

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The cardiodynamic and renal changes in chronic pericarditis with effusion were investigated in the dog with particular reference to mechanisms of fluid retention in relation to congestive heart failure.

Pericarditis was induced with irritative cellophane. Post-mortem morphologic studies revealed chronic nonbacterial pericarditis with effusion, passive hyperemia of the liver, kidneys, and lungs, and anasarca.

The following determinations were done serially on unanesthetized dogs: (1) cardiac output (C.O.), (2) renal plasma flow (R.P.F.), (3) glomerular filtration rate (G.F.R.), (4) Na clearance, (5) plasma volume, (6) thiocyanate space, (7) hematocrit, (8) plasma proteins, (9) central and peripheral venous pressure (V.P.), (10) arterial blood pressure, (11) heart rate, (12) intrapericardial pressure, (13) weight.

As pericardial effusion developed, peripheral and central venous pressure rose. This was the initial change recorded. No dog had an increased plasma volume at this time. In some animals thiocyanate space was normal, in others it was moderately increased. Resting C.O., R.P.F., and G.F.R. were all normal at this time. Na clearances suggested slightly decreased Na excretory rate.

With progression of tamponade, V.P. rose further. Plasma volume was increased in some animals, at control level in others. Thiocyanate space was elevated. In this late phase, resting C.O., G.F.R., and R.P.F. were at control levels. Arteriovenous O_2 difference was increased. Ability to increase C.O. in response to excitement and exertion was severely limited. Blood pressure and pulse pressure were moderately depressed.

Prior to exitus, circulatory collapse occurred, with hypotension, markedly elevated V.P. and intrapericardial pressure, decreased right atrial effective filling pressure. Resting C.O., R.P.F., and G.F.R. were depressed. Na clearance was severely impaired, with prolonged retention of infused saline.

It is concluded from this study that in pericarditis with tamponade: (1.) Venous pressure rises as increased intrapericardial pressure jeopardizes right atrial effective filling pressure. Increased venomotor tone appears to be a key factor in the V.P. rise.

(2.) Elevated hydrostatic pressure leads to edema formation. This occurs despite normal resting C.O., G.F.R., R.P.F., and plasma volume.

(3.) It is suggested that with elevated renal venous pressure, increased tubular Na reabsorption brings about Na and H_2O retention. Plasma volume is thereby maintained despite increased hydrostatic pressure; edema develops without hemoconcentration. Inadequate C.O. during activity may also contribute to Na retention due to an inordinate fall in R.P.F. and G.F.R.

(4.) Preterminally, a critical level of intrapericardial pressure is reached (10 to 15 cm. of H_2O) beyond which V.P. cannot rise to maintain right atrial effective filling pressure and C.O. Circulatory collapse ensues. All mechanisms for Na and H_2O retention are aggravated. Exitus soon supervenes.

27. CLINICAL AND EPIDEMIOLOGIC STUDIES OF MUMPS EMPLOYING THE COMPLEMENT FIXATION TEST

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The complement fixation test has been employed for a clinical and epidemiologic study of mumps. Data have been obtained concerning the persistence of antibodies following infection, the occurrence of unrecognized infections, the diagnosis of suspected cases of mumps and of "aseptic meningitis," and a comparison of the "S" or soluble antigen, and the "V" or virus antigen. The results confirm and extend data presented previously from other laboratories.

Tests on one hundred fifteen single sera employing infected allantoic fluid ("V" antigen) detected antibody in the sera of fifty of fifty-one individuals who gave a definite past history of mumps. This positive correlation is notable because the majority of these persons had had mumps ten to thirty years previously. There were seventeen individuals whose sera contained no detectable antibody and it seems highly significant that fourteen of them had no history of mumps, two had an equivocal history, and only one had had mumps. Sera from thirty-two, or 70 per cent, of forty-six individuals with a negative past history of mumps contained antibody, indicating that inapparent or mild infections are frequent.

Diagnostic tests were made with acute phase and convalescent phase sera from thirty-four patients, with definitive results. Twelve sets of sera showed large increases in titer; eleven of these were from cases of mumps, many with complications, and the twelfth was from a patient in whom mumps was strongly suspected. Twenty-two sets showed no significant change in titer; all were from patients in whom mumps was considered in the differential diagnosis and thirteen were from patients with "aseptic meningitis."

Tests employing "S" antigen indicated, as Henle has reported, that antibody to this antigen usually does not persist as long as antibody to the "V" antigen. "S" antigen afforded no decided advantage over "V" antigen for the detection of mumps with paired acute and convalescent phase sera.

It is concluded that the complement fixation test is a valuable tool for clinical and epidemiologic studies of mumps. In addition, the test has been of practical clinical importance in certain instances, such as in patients exposed during early pregnancy and in professional personnel.

28. DETERMINATION OF TOTAL BODY SODIUM IN MAN WITH RADIOSODIUM[24]

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Previous methods for determining the sodium content of the human body have been based, for the most part, on calculations from chemical analyses of various organs and an assumed ratio of these organs to the total mass of the

body. Carcass analysis is obviously more accurate, yet reports of such analyses are available only for the fetus and newborn. Since knowledge of the sodium content of the body should be useful in estimating parenteral fluid requirements, an attempt was made to determine it by the isotope dilution principle.

Intravenously administered radiosodium quickly mixes with intravascular sodium and within two to three hours is in equilibrium (except for cerebrospinal fluid) with extravascular sodium. Equilibration with the sodium of brain and bone takes place more slowly but is believed to be complete in about eighteen hours. When equilibrium is complete, serum specific activity should equal total body specific activity, and from knowledge of the former, total body sodium can be calculated as follows:

$$\frac{Na^{24} \text{ injected} - Na^{24} \text{ excreted}}{\text{Serum } Na^{24}/\text{serum } Na^{23}} = \text{Total body } Na^{23}$$

Thirty determinations on twenty-seven healthy young men indicate that total body sodium has an average value of 41 meq. per kilogram. In a series of seventeen children the average values are 43 meq. per kilogram for the older children, 49 meq. per kilogram for large infants, and 82 meq. per kilogram for small infants. Comparison of these results with those of actual chemical analyses from the literature will be made.

29. COMPARISON OF THE ELECTROPHORETIC PATTERN OF SERUM AND PLASMA IN LIVER DISEASES WITH SPECIAL REFERENCE TO THE GAMMA GLOBULIN FRACTIONS

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Electrophoretic studies on patients with chronic liver disease revealed marked elevations of the fibrinogen peak and lower than expected levels of gamma globulin. To study this phenomenon, we made a comparative study of sixty-five pairs of plasma and serum from persons having various liver diseases, a miscellaneous disease group and a normal group. Electrophoretic studies were supplemented by chemical protein partition (Wolfson and Cohn), flocculation, and other hepatic tests in all except a few miscellaneous cases.

Chemical analysis did not agree with the marked electrophoretic elevations in fibrinogen, nor were fibrinogen peaks completely obliterated upon electrophoretic analysis of the serum. Total serum gamma globulin was higher than the apparent plasma electrophoretic gamma globulin. The serum gamma globulin appeared electrophoretically as two portions of different mobilities, one migrating in the fibrinogen range. This latter portion accounted for the difference between the serum and plasma gamma globulin and, having a similar mobility to fibrinogen, was buried in that peak in plasma determinations. This $gamma_1$ fraction added to the plasma gamma globulin gave values similar to those of serum and chemically determined gamma globulin. It also accounted for most of the abnormal electrophoretic fibrinogen elevation and when subtracted from the apparent fibrinogen, chemical fibrinogen values were approached. This gamma fraction is apparently identical to the gamma fraction found by Deutch in normal individuals. In our normal group it formed

approximately 2.4 per cent of total serum protein and 16.1 per cent of total gamma globulin. In acute hepatitis it was somewhat higher than normal, averaging 3.9 per cent of total serum protein and 16.7 per cent of total gamma globulin. In obstructive jaundice it was 4.1 per cent and 24.0 per cent, respectively. In cirrhosis this gamma globulin fraction averaged 7.0 per cent of total serum protein and 22.7 per cent of total gamma globulin. Thus, in chronic liver disease in addition to a total gamma globulin increase, there is a disproportionate increase in the gamma$_1$ fraction. Fifteen cases of rheumatoid and one multiple myeloma showed expected total gamma globulin increases but little change in the gamma$_1$ fraction. The serum and plasma concentrations of albumin, alpha and beta globulins, were essentially similar. Although plasma electrophoretic tracings appear to be more characteristic for chronic liver disease than do those of serum, their use in protein partition of liver disease is limited because their inability to separate components of the elevated fibrinogen peak gives false impressions of elevated fibrinogen values and lower than expected globulin values.

30. INTRA-AORTIC BLOOD PRESSURE DURING SURGICAL RESECTION AND REPAIR OF COARCTATION OF THE AORTA

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(INTRODUCED BY HOWARD B. BURCHELL, M.D.)

A mobile oscillographic camera was used during nine operations for coarctation of the aorta to record continuously the electrocardiogram, heart rate, respiration, and direct blood pressure in the right radial artery. The pressure in the aorta above and below the stricture was recorded by direct puncture simultaneously with these other variables before and after repair of the coarctation.

Compression of the area of the stricture produced no signicant change in the pressure in the radial artery or in the aorta, whereas compression of the left subclavian artery in four patients produced an average increase of 18 mm. Hg in the systolic and pulse pressures in the radial artery and an increase of 17 mm. Hg, systolic, and 15 mm. Hg in pulse pressure in the aorta above the stricture. In spite of an increased pressure above the stricture, this maneuver in the three cases studied produced an average decrease in the pressure in the aorta below the stricture of 7 mm. Hg, systolic, and 5 mm. Hg, mean.

In eight patients the average time spent opening the anastomosis was 31 seconds, and during this time the mean pressure in the radial artery decreased an average of 11 mm. Hg.

Repair of the coarctation in six patients was associated with an increase of 34 mm. Hg, systolic, and 22 mm. Hg, pulse pressure, in intra-aortic pressure below the stricture.

In seven patients the onset of the pulse wave in the aorta distal to the stricture before repair was an average of 0.02 second later than in the right radial artery at the wrist, whereas after resection the onset of the pulse distal to the site of stricture preceded that of the wrist by an average of 0.05 second. This was not signicantly different from the value obtained from the aorta above the site of stricture.

Correlation between (1) the pressures at operation and (2) preoperative and postoperative direct observations of radial and femoral arterial pressures was not sufficiently close to be evident in this study.

The dynamic responses of the strain gage manometer systems used were studied by measurement of their response to square wave and variable frequency sine wave pressure variations. The response to equal amplitude pressure variations was within ±8 per cent of the true pressure change up to 30 c.p.s. Higher frequencies were recorded with diminished sensitivity. The deflection time was 0.02 second and the damping coefficient was 0.62. These dynamic characteristics are superior to those of undamped manometers with resonant frequencies of 150 c.p.s.

31. THE EFFECT OF CHOLESTEROL-FREE DIET ON SERUM CHOLESTEROL OF NORMAL AND THIOURACIL-TREATED DOGS

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Experimental studies in dogs indicate that altered thyroid function is necessary for the production of atherosclerosis by cholesterol feeding. In order to elucidate the importance of dietary cholesterol, the following experiment was devised.

Weekly weight and serum cholesterol determinations were made on two groups of four dogs each. After a control period of ten weeks on a commercial dog food, both groups were placed on a diet composed entirely of vegetable products. After a second control period of eight weeks, blood samples were drawn from the hepatic vein and right auricle by means of the catheter technique. Animals in group 2 were then given 1.0 Gm. thiouracil daily. The dose was increased to 1.5 mg. at twenty-five weeks.

In two dogs of group 2, after serum cholesterol had increased to twice the highest control value, hepatic vein and right auricular blood was analyzed for cholesterol.

In group 1, average serum cholesterol was 210 mg. per cent for one dog and 130 mg. per cent for the remaining three animals. This fell to an average of 176 mg. per cent for Dog 1 and 100 mg. per cent for the others. The fall was steady and progressive, lowest levels occurring toward the end of the period.

Group 2 showed striking increase in serum cholesterol after being given thiouracil for ten days. The serum cholesterol reached an average level of 240 mg. per cent over a period of eleven weeks. This approximately doubled average control values.

Comparison of mixed venous blood from the right auricle and hepatic vein showed no significant difference in cholesterol content before and after thiouracil feeding.

It is concluded that (1) the dog can be maintained in good condition on a cholesterol-free diet; (2) all dogs maintained on such a diet show gradual diminution of serum cholesterol; (3) elevation of serum cholesterol produced by thiouracil is not dependent on exogenous cholesterol; (4) excessive production of cholesterol by the liver was not demonstrated in a limited number of animals; (5) the possibility that shift of cholesterol from various organs to the blood serum occurs is being further investigated.

32. THE RENAL CAPACITY OF NORMAL, HYPERTENSIVE, AND CARDIAC FAILURE PATIENTS TO EXCRETE SODIUM

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The capacity to excrete an intravenous dose of 20 Gm. of sodium chloride per 70 kilograms of weight was studied in normal, hypertensive, and cardiac failure patients. A salt-free diet and distilled water were given one day before and for the two days of the experiment. The sodium content of two-hour urine specimens was determined for the first twelve hours and for three additional twelve-hour periods for a total of forty-eight hours following the injection of sodium chloride. These studies were done on eight normal, nine hypertensive, and eight heart failure patients. The hypertensive patients were studied only if venous pressure, kidney concentration tests, P.S.P. tests, and blood nonprotein nitrogen showed no evidence of renal failure.

Marked impairment of the ability of the kidney of each patient with cardiac failure and slightly less impairment of the kidney of each patient with hypertension to excrete sodium was observed in every one of the periods studied.

33. PRELIMINARY REPORT OF EXPERIENCES WITH Rh HAPTEN

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(INTRODUCED BY MAURICE HARDGROVE, M.D.)

Hapten is the term introduced by Landsteiner to designate that portion of the antigen complex which determines its specific reactivity. Separated from the protein portion of the antigenic substance, the haptenic fraction is incapable of calling forth an antibody response when injected into an animal but retains its ability to react with and bind its specific antibody in vitro. We have attempted to extract the haptenic portion of the Rh agglutinogen from Rh-positive red blood cells with alcohol and ether, using a modification of the procedures described by Carter and Price. This material was assayed in vitro by complement fixation, using the method described by Kolmer for the serodiagnosis of syphilis. The hapten material obtained was injected into patients with demonstrable anti-Rh antibodies during pregnancy; 100 to 200 mg. of the crude material was injected intramuscularly at weekly intervals.

Sixteen of our patients have terminated their pregnancies. Five received eight or more injections and eleven less than eight injections. Of the five receiving "adequate" therapy, two delivered rh-negative babies, two had macerated, stillborn fetuses at thirty-two to thirty-four weeks, and one delivered an Rh-positive infant with demonstrable antibodies coating its red cells but no clinical evidences of erythroblastosis fetalis. Eleven patients were seen too late and received only 2 to 6 injections of hapten. Two of these delivered macerated, stillborn fetuses, one aborted at eleven weeks, one infant was rh negative, two survived after multiple transfusions, two survived after replacement transfusions, and three showed no clinical or hematologic involvement although they were Rh positive and had positive Coomb's tests.

Definite conclusions cannot be drawn from this material. The anti-Rh titers of our patients receiving hapten have tended to remain low. In spite of this, our results have been discouraging. For example, one patient delivered a macerated fetus during the thirty-fourth week of pregnancy after receiving seventeen injections of hapten. Her titer had never risen above 8 in albumin

and was only 2 three days before delivery. Another patient who had only four hapten injections delivered a normal Rh-positive infant even though antibodies could be demonstrated coating its red cells. One must almost conclude that the hapten therapy was not the determining factor.

34. CARDIOVASCULAR LESIONS IN RATS SUBJECTED TO GROUP A BETA HEMOLYTIC STREPTOCOCCAL PULMONARY INFECTIONS

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The effect of repeated pulmonary streptococcal infections in the rat was investigated in an attempt to elucidate the nature of the relationship of group A beta hemolytic streptococcal infections to rheumatic fever.

Pneumonia was induced in albino rats by intrabronchial inoculation of group A beta hemolytic streptococci employing techniques previously described. Animals were subjected to from one to seven infections; two strains of streptococci were used alternately in the animals infected repeatedly. Beginning eighteen hours after inoculation, animals were treated with penicillin in amounts previously determined to be adequate for successful control of the infection.

Infections were produced usually at two-week intervals; animals were sacrificed at the end of given periods for study. The hearts were fixed in Zenker-formalin, and microscopic sections were stained with hematoxylin-eosin.

Slight to moderate arteritis and periarteritis of the coronary and/or myocardial arteries occurred in 30 per cent of the experimental animals, whereas 3 per cent of the normal controls exhibited this change. The lesions observed in the experimental group were more intense than those in the control group.

Myocardial lesions, consisting chiefly of pleomorphic cellular foci, were noted in 52 per cent of the animals repeatedly infected; 38 per cent of the controls had similar but less intense lesions.

The incidence of endocardial infiltration by mononuclear cells was slightly more frequent and more intense in the experimental animals than in the controls. Cellular infiltration within the valve substance itself, however, was noted with greater frequency in the control animals. The cardiac lesions observed did not resemble those of rheumatic fever and none of the animals developed arthritis.

Results of this study have demonstrated a significantly greater incidence of cardiac arterial lesions in rats subjected to repeated pulmonary infections with group A beta hemolytic streptococci than in normal control subjects. Single streptococcal infections were not identified with a higher incidence of cardiovascular changes than were found in normal subjects.

The failure of the rat to develop more striking morphologic manifestations of hypersensitivity is in keeping with previous observation. It is believed, however, that the experimental approach used in this investigation, applied to other animal species, may prove a valuable means of studying the problem of the relation of streptococcal infection to rheumatic fever.

35. OBSERVATIONS OF THE CHARACTER OF PLATELETS STUDIED WITH A NEW PHOTOGRAPHIC TECHNIQUE

F. R. HALL, M.D. (BY INVITATION), AND STUYVESANT BUTLER, M.D., CHICAGO, ILL.

In previous reports of Butler, Thomas, and Sanford it was observed that in patients receiving intravenous histamine there was no decrease in the number of platelets, but there did occur a slight but definite decrease in the clotting time

of platelet-free plasma; the higher the histamine concentration, the greater the decrease. It was also observed by Sanford, Butler, and Kennedy that the clotting time of children with hemophilia could be greatly reduced by intravenous histamine.

In order to correlate these observations with the disappearance of platelets and increased coagulation in shock, we surmised that there must be a simultaneous increased production and destruction of platelets at these clinical levels. To study this theory we have given intravenous histamine to patients using techniques described for coagulation studies, but for absolute accuracy, convenience, and a permanent indisputable record, we have made serial photographs every three minutes of platelets in solution for many hours.

Platelets examined in this manner can be studied morphologically with great accuracy and appear to remain constant in number for a long time, and after a period of settling are found to be of a uniform size and characteristic spindle shape.

Conclusions.—(1) Platelets are a formed element of the blood. (2) Platelets in solution for many hours in contact with glass do not change their character. (3) A photographic technique for the accurate study of platelets is described.

36. THE DIFFERENTIAL DIAGNOSIS OF HYPERGLYCEMIC STATES BY LABORATORY METHODS

G. Hamwi, M.D. (By Invitation), and E. von Haam, M.D., Columbus, Ohio

The hyperglycemic state represents a symptom and not a disease and can be produced by various etiological factors. In twenty-three patients in whom a disturbance of carbohydrate metabolism was suspected the glucose tolerance and the insulin glucose tolerance were studied in conjunction with other supplementary laboratory tests. The arterial-venous difference in the glucose tolerance test was taken as evidence of peripheral glucose utilization. Since this utilization is influenced by insulin, a normal arterial-venous difference would imply normal insulin effect. Decreased insulin sensitivity was recognized in the insulin glucose tolerance test. It was taken as evidence of the presence of extrapancreatic factors influencing the carbohydrate metabolism. Using these criteria, seven of the twenty-three cases were recognized as having a normal carbohydrate metabolism. The sixteen patients suffering definitely from hyperglycemia could be divided into the following groups: 4 cases of true pancreatic deficiency hyperglycemia, 3 cases of hyperglycemia showing pancreatic deficiency and extrapancreatic factors, 4 cases of extrapancreatic hyperglycemia, and 5 cases of hyperglycemia with insulin and insulin sensitivity. The latter group may include the nutritional, hepatogenous, and mild functional hyperglycemias.

The hypothesis is presented that patients suffering from carbohydrate metabolism disturbance may change from one type to another, thereby either aggravating or improving the hyperglycemic state. Some confirmation of the foregoing classification was found in the supplementary laboratory evidence and the clinical data available. Patients in whom extrapancreatic influences were postulated as a factor in the hyperglycemia suffered either from obesity or from other demonstrable functional disorders. The possibility that nonglucose reducing substances may interfere with a correct interpretation of the arterial-venous differences is discussed and illustrated with a pertinent case.

37. THE EFFECT OF 4-AMINO-PTEROYLGLUTAMIC ACID ON THE URINARY EXCRETION OF 17-KETOSTEROIDS AND CORTICOSTEROIDS IN ACUTE LEUCEMIA

DAVID G. HANLON, M.D. (BY INVITATION), HAROLD L. MASON, PH.D., AND J. M. STICKNEY, M.D. (BY INVITATION), ROCHESTER, MINN.

Dougherty and Higgins, working independently, observed atrophy of the thymus, spleen, and lymphoid tissues and hyperplasia of the adrenal cortex after administration of toxic doses of 4-amino-pteroylglutamic acid (Aminopterin) to rats. Adrenalectomy largely prevented the involution of lymphoid structures. These findings suggested that Aminopterin exerts its effect, in part at least, through the medium of the adrenal cortex and led us to study adrenal function by means of determinations of the urinary excretion of corticosteroids and 17-ketosteroids of patients with acute leucemia who were being treated with Aminopterin.

In a control series of eighteen patients who had untreated acute leucemia, the values for urinary excretion of corticosteroids were usually within normal limits for adults (0.3 to 1.0 mg. in twenty-four hours); in one patient the value was less than normal and in three patients slightly more than normal. Low or low normal values (0.8 to 5.5 mg. in twenty-four hours) were found for 17-ketosteroids.

Six patients having acute leucemia were treated with 0.5 to 1.0 mg. of Aminopterin daily; there was no evidence of increased urinary excretion of corticosteroids or 17-ketosteroids. In four of the six patients the output of corticosteroids in urine was depressed significantly, and in two of these four it practically disappeared. In the remaining two of the six patients there was no appreciable change from the original values, despite the administration of a total of 23 and 9 mg., respectively, of Aminopterin.

Depression of the urinary excretion of corticosteroids after administration of Aminopterin may result from one or more of three possible causes: (1) cytotoxic effect of Aminopterin on the adrenal cortex with reduced activity of this gland; (2) a toxic effect on the pituitary with failure of its adrenocorticotropic function; and (3) altered metabolism of cortical hormones.

Administration of 25 mg. of pituitary adrenocorticotropic hormone to one patient who was excreting subnormal amounts of corticosteroids was followed by no change in the number of circulating eosinophils and only a 3 per cent increase in the ratio of uric acid to creatinine, which, according to the experience of Thorn, would indicate impaired adrenocortical function. Nevertheless, in response to the adrenocorticotropic hormone there was a marked rise in the output of corticosteroids which showed that the adrenal cortex was still capable of responding to pituitary adrenocorticotropic hormone and suggests that Aminopterin may effect a depression of the adrenocorticotropic function of the pituitary.

38. HEMORRHAGIC DIATHESIS ASSOCIATED WITH LOW THROMBOPLASTIC ACTIVITY AND A CIRCULATING ANTICOAGULANT

ROBERT W. HEINLE, M.D., AUSTIN S. WEISBERGER, M.D. (BY INVITATION), PAUL J. VIGNOS, M.D. (BY INVITATION), AND WILLIAM B. HOLDEN, M.D. (BY INVITATION), CLEVELAND, OHIO

An acquired hemorrhagic disease occurring in a woman one year after a normal pregnancy and characterized by a prolonged coagulation time, the presence of a circulating anticoagulant, and an apparent deficiency of thromboplastin

has been studied over a period of six years. The hemogram, platelet count, bleeding time, clot retraction, tourniquet test, prothrombin level, plasma fibrinogen, serum calcium, plasma vitamin C, and plasma proteins have all been within normal limits on repeated determinations. The administration of large amounts of both fresh whole blood and plasma has not had any effect on the prolonged coagulation time. Precipitins against plasma fractions I and III have not been demonstrable. There was no change in the clotting time of recalcified plasma following centrifugation at high and low speeds. Titration with protamine sulfate revealed no increase in heparin. The presence of a circulating anticoagulant was demonstrated by the prolongation of the clotting time of normal bloods when serial amounts of the patient's blood were added. The anticoagulant effect was also demonstrable in the plasma. Heating at 60° C. for ten minutes inactivated the anticoagulant effect of the plasma. Standing at room temperature or 4° C. for forty-eight hours had little or no effect on the anticoagulant. Thromboplastin assay of the patient's plasma compared with normal plasma and a standard preparation of Maltine thromboplastin revealed low levels of available thromboplastin. Plasma staphylococcus coagulase activator was normal. On two occasions electrophoretic studies revealed the presence of an abnormal fast component of the albumin fraction of the serum. Administration of a course of nitrogen mustard to the patient did not alter the clinical or laboratory findings.

The defect in coagulation in this patient is the result of the presence of an abnormal circulating anticoagulant. The findings do not differentiate whether the mechanism involves (1) antagonism of thromboplastin, (2) antagonism of a precursor of thromboplastin, or (3) an antienzyme antagonistic to a platelet enzyme necessary for the conversion of thromboplastin precursor to thromboplastin. The end result, however, is a deficiency of thromboplastic activity in the blood which produces hemophilic type of bleeding in the patient.

39. CONTROL OF HEART RATE WITH AN INTRACARDIAC THERMODE

HERMAN K. HELLERSTEIN, M.D., AND IRVING M. LIEBOW, M.D., CLEVELAND, OHIO

(INTRODUCED BY HAROLD FEIL, M.D.)

Classical experiments of McWilliam, Flack, and others have shown that the rhythmicity of the S-A node can be altered by thermal changes in excised and perfused hearts and in the exposed heart. We have devised a special thermode which can be placed in the region of the S-A node by venous (jugular, brachial) catheterization of the *intact* experimental animal.

The thermode consists essentially of a 4 × 30 mm. copper U tube attached to the cardiac end of a double lumen catheter. The thermode temperature is regulated by perfusing water of various temperatures (4 to 60° C.) through this closed system. In five Nembutalized, intact dogs, changes in heart rate and in the form of the electrocardiogram were determined by continuous tracings recorded before, during, and after thermal changes. In over 100 experiments, it was possible to vary the rate at will, similar to experiments on exposed hearts.

The most pronounced effects occurred when the thermode was located at the junction of the SVC and right atrium, in the region of the S-A node. There was a latent period of two to twenty-five seconds. Changes in rate were considered to be due to alteration of the temperature of the region of the S-A node. Cold perfusion caused the sinus rate to decrease from the control level of 120 to 140 to 90 to 80 per minute. When the S-A node region was cooled excessively, the pacemaker shifted to the A-V node, with a rate varying from 57 to 86 per

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The most pronounced effects occurred when the thermode was located at the junction of the SVC and right atrium, in the region of the S-A node. There was a latent period of two to twenty-five seconds. Changes in rate were considered to be due to alteration of the temperature of the region of the S-A node. Cold perfusion caused the sinus rate to decrease from the control level of 120 to 140 to 90 to 80 per minute. When the S-A node region was cooled excessively, the pacemaker shifted to the A-V node, with a rate varying from 57 to 86 per

minute. Hot perfusion increased the sinus rate to a maximum of 200 to 232 per minute at 45 to 55° C. The acceleration produced by heating was relatively greater and persisted longer than the slowing produced by cooling.

Marked changes in rate occurred without T-wave alteration. However, in some experiments, when perfusion was rapid, there were T-wave changes which indicated that there had been sufficient temperature change of the blood passing the thermode to alter the rate of repolarization of the subendocardial lamina. Thus, in accordance with our previous observations, cold perfusion produced large negative T waves in cavitary leads and tall positive T waves in extracavitary leads; heating produced opposite changes.

The intracardiac thermode may prove valuable experimentally and therapeutically.

40. THE EFFECT OF RUTIN ON THE HEMORRHAGIC PHENOMENA OF EXPERIMENTAL MALIGNANT HYPERTENSION IN THE DOG

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A striking feature of the malignant phase of experimental Goldblatt hypertension is the occurrence of diffuse hemorrhages. The cause of these hemorrhages is unknown, usually being attributed to degeneration and necrosis of arterioles and capillaries.

In view of the contradictory reports concerning the effects of rutin in reducing capillary fragility and hemorrhage in the malignant phase of hypertension in man, the following study was undertaken. Acute hypertension with uremia was produced by complete bilateral ligation of the renal arteries in sixteen dogs. Rutin was administered subcutaneously, 200 mg. per day, as indicated in Table I.

Table I

Number of dogs	Rutin received		Hemorrhagic lesions
	Preoperatively	Postoperatively	
5	0	0	Severe
2	0	Daily	Severe
4	3 days	Daily	Severe
5	10 days	Daily	None

All the animals developed clinical uremia and hypertension, with death in three to six days. The tissues were studied as unknowns.

Severe hemorrhages in the gastrointestinal tract, heart, pancreas, urinary bladder, diaphragm, spleen, and adrenals, together with myocardial inflammation and necrosis, were seen in the first three groups. By contrast, the animals pretreated with rutin for ten days showed complete absence of cardiomyopathy and of the hemorrhagic changes. As a result of the complete obstruction of the main renal arteries, coagulation necrosis of the renal cortex and medulla, with an unexpected sparing of the corticomedullary junction, was noted.

These experiments demonstrate that the hemorrhages usually seen in acute experimental malignant hypertension were prevented by pretreatment with rutin for ten days prior to operation. This protective action may be due to a stabilizing effect of rutin on the ground substance of the arterioles and of the pericapillary sheath. The fact that a shorter period of pretreatment did not provide protection suggests that all our animals were relatively rutin deficient

and that saturation required more than three days of pretreatment. The contradictory reports on the effect of rutin in malignant hypertension in man may also depend upon the relative rutin deficiencies in the patients studied. These experiments call attention to the possibility that the several dietary regimens now being used in the treatment of hypertension may be deficient in rutin and possibly other metabolites.

41. A CLINICAL EVALUATION OF THE BLOOD "SLUDGE" PHENOMENON

JOHN S. HIRSCHBOECK, M.D., AND MAYO WOO, M.D. (BY INVITATION), MILWAUKEE, WIS.

The bulbar conjunctival capillaries of a variety of medical patients were observed with the capillary microscope. Erythrocyte sedimentation rates, erythrocyte counts, and serum protein determinations were made within a few days before or after the observation of the circulation in the capillaries. More than 1,200 observations were made.

In general, the degree of "sludging" was directly proportional to the sedimentation rate. "Sludging" was observed in a wide variety of pathologic conditions. Patients with hyperglobulinemia usually had an increased sedimentation rate and well-developed "sludge." Anemia usually caused an increase in "sludging" and sedimentation, whereas erythrocytosis (polycythemia vera or congenital heart disease) always produced an opposite effect.

A discrepancy between the degree of "sludging" and the sedimentation rate occurred in 17.6 per cent of the 619 cases studied. In 5.5 per cent of the cases there was a minimal degree of "sludging" with a rapid sedimentation rate. Pneumonia was the most frequent diagnosis in this group. In 12.1 per cent of the cases well-developed "sludging" with a slow sedimentation rate occurred. Heart failure and hepatic cirrhosis were the most frequent diagnoses in this group. Well-developed "sludging" in the absence of a rapid sedimentation rate is probably the result of stasis in the capillaries.

These observations indicate that blood sedimentation and blood "sludging" have a common cause which, according to Fahraeus and others, is increased rouleaux aggregation.

42. FURTHER EXPERIENCES IN THE MANAGEMENT OF LOWER NEPHRON NEPHROSIS

WILLIAM S. HOFFMAN, M.D., ARTHUR BERNSTEIN, M.D. (BY INVITATION), LIONEL BERNSTEIN, M.D. (BY INVITATION), AND PHILIP B. O'NEILL, M.D. (BY INVITATION), CHICAGO, ILL.

Lower nephron nephrosis was found to occur after a variety of shocklike episodes, only a few of which were associated with muscle injury or destruction of blood cells. It was seen in lobar pneumonia, acute pancreatitis, acute enteritis, and diabetic coma. The condition was at times difficult to distinguish from other diseases producing oliguria and hematuria, such as acute glomerulonephritis, and bilateral papillary necrosis. Factors producing rapid dehydration and sodium loss, which ordinarily lead to so-called extrarenal azotemia and which respond to fluid and salt administration, could in more fulminating episodes produce true lower nephron nephrosis. The ultimate clinical diagnosis in the patients who survived rested on the finding of severe oliguria from five to thirteen days unresponsive to management, with spontaneous and progressively

increasing diuresis, and with poor renal function even after subsidence of blood nitrogen levels, which function slowly but ultimately returned to normal.

The original illness which brought on the lower nephron nephrosis was important in the prognosis. If infection or trauma persisted throughout the period of oliguria, tissue breakdown was excessive and blood nitrogen levels rose precipitously. These patients died in uremia and heart failure under conditions in which previously well persons might easily survive. All five deaths in our series of eighteen patients occurred with such complications. All patients whose oliguria was associated with self-limiting or controllable conditions, like that of hemorrhage, transfusion reaction, or controllable infection, survived.

We continue to advocate only conservative management in these cases. In the first six cases, successful resistance to the ravages of uremia was achieved by slow induction of edema of nearly normal electrolyte composition. In the later cases, the same results were accomplished by raising sodium levels to normal by intravenous injections of small quantities of 3 per cent sodium chloride and by oral sodium bicarbonate. Thus edema was kept at a minimum. We continue to allow a soft diet ad libitum and believe the slight increase in blood nonprotein nitrogen concentration and edema thereby produced less harmful than the weakness, thirst, and anxiety produced by water and food deprivation advocated by others. Watchful symptomatic treatment of the emergencies of uremia still remains an important factor in successful management.

43. TURBIDIMETRIC DETERMINATION OF SERUM GAMMA GLOBULINS AS CHECKED BY ELECTROPHORETIC ANALYSIS

J. DE LA HUERGA, M.D. (BY INVITATION), AND HANS POPPER, M.D., CHICAGO, ILL., AND MURRAY FRANKLIN, M.D. (BY INVITATION), IOWA CITY, IOWA

Recently a simple method for turbidimetric estimation of the gamma globulins was described by Huerga and Popper. It follows closely the procedure of the thymol turbidity test and is based on recording of a precipitate which forms if serum is diluted with an ammonium sulfate-sodium chloride solution, simulating the chemical determination and partition of gamma globulins by Wolfson, Cohn, and others. The results of the turbidimetric method were found identical with this chemical method. The latter is supposed to give comparable results with electrophoretic partition. Nevertheless, the direct results of the turbidimetric readings for gamma globulins were compared with the results of electrophoretic partition in 163 blood specimens of 134 cases which included normal subjects, patients with various liver diseases, multiple myeloma, and miscellaneous disorders. In 58.1 per cent of the cases the difference between the results of the electrophoretic determination in plasma and the turbidimetric in serum was less than 15 per cent; in 71.5 per cent, less than 20 per cent. The results were not comparable at all in only 3.7 per cent of the cases, varying more than 50 per cent. The mean difference between the turbidimetric and electrophoretic method in per cent was 17.0 ± 15.3, the electrophoretic method giving slightly higher values on the average. In thirty-seven cases in which both determinations were performed in serum, the mean difference was 13.5 ± 8.4 per cent. The differences were not more marked in pathologic conditions than under normal circumstances. This comparison indicates that the simple gamma globulin turbidity test may replace the electrophoretic determination of gamma globulins in clinical use. The results of the gamma globulin turbidity test are not identical with those of the zinc sulfate turbidity test of Kunkel, especially in hepatic disorders, since the latter may appear depressed in intra- and extrahepatic obstructive jaundice. This depression indicated by an abnormally low ratio between zinc sulfate and gamma globulin turbidity may therefore have diagnostic sig-

nificance. The normal gamma globulin turbidity values range between 0.60 and 1.25 Gm. per 100 ml. of serum. Any elevation above this level is significant. The gamma globulin turbidity values are markedly elevated in liver disorders, especially in cirrhosis and viral hepatitis, and also in rheumatic diseases, several forms of chronic tuberculosis, collagen diseases, chronic skin diseases, and other chronic infections. Comprehension of the exact diagnostic value of elevation of the gamma globulin turbidity in the latter conditions requires additional investigation.

44. AN UNUSUAL CLINICAL PICTURE RESEMBLING PROLONGED SERUM SICKNESS "THOUGHT TO BE CAUSED BY TRICHINOSIS"

JOHN S. HUNT, M.D., NEW ORLEANS, LA.

A young Negro laborer, previously well except for nonseasonal attacks of asthma since childhood, was hospitalized with an illness of one month's duration, characterized by an abrupt onset of fever, migratory arthritis, and severe pain and tenderness in the leg muscles. He had received no serum or drugs prior to this illness. When first seen, the muscle pain had largely subsided, but he had persistent fever and arthritis, and physical examination revealed, in addition, pronounced enlargement of subcutaneous lymph nodes. There was no rash or demonstrable edema. The clinical picture suggestive of serum sickness was supported by laboratory observations of marked leucocytosis; eosinophilia of 4 to 8 per cent; marked elevation of serum gamma globulin, as evidenced by the zinc sulfate turbidity test; and the presence of serum heterophile agglutinins, of the Forssman type, in rising titer. Lymph node biopsy in the fifth week of illness showed marked hyperplasia, and muscle biopsy in the sixth week revealed no trichinae or arteritis. There was a strongly positive, immediate reaction to dilute trichina antigen injected intradermally. An electrocardiograph showed changes in T waves and QRS complexes suggestive of myocardial disease. Fever, leucocytosis, and joint pains slowly subsided over a six-week period, and there was a gradual decrease in the size of the lymph nodes. Pyribenzamine treatment during the stage of acute illness resulted in a slight decrease in fever and a definite improvement in the arthritis, pain, and objective joint changes decreasing promptly and recurring explosively, on two occasions, after withdrawal of the drug.

On re-examination, eight months after the onset of the illness, he was asymptomatic and showed, on examination, nothing abnormal except for palpable lymph nodes of much smaller size. The leucocyte count was normal, but with 4 to 5 per cent eosinophiles. Heterophile agglutinins had disappeared from the serum. The electrocardiograph now showed a P-R interval of 0.22 second.

Trichinosis is suggested as the probable cause of this illness on the basis of the muscle pain and tenderness at its onset, the strongly positive intradermal test with trichina antigen, the persistent eosinophilia, and the electrocardiographic abnormalities. This suggests the possibility that, on occasion, the clinical picture of trichinosis may be chiefly one of hypersensitivity to the trichina antigen. In this connection, the report of Reimann, Price, and Herbut on the association of trichinosis with lesions of periarteritis nodosa is of interest.

45. FLUID AND ELECTROLYTE BALANCE IN THE MANAGEMENT OF ACUTE RENAL INSUFFICIENCY

LLOYD T. ISERI, M.D. (BY INVITATION), ALBERT J. BOYLE, PH.D., M.D. (BY INVITATION), THOMAS M. BATCHELOR, M.D. (BY INVITATION), SAMUEL D. JACOBSON, M.D., AND GORDON B. MYERS, M.D., DETROIT, MICH.

Studies of Na, K, Cl, N, and water balance were carried out for periods of four to sixteen days in four patients with severe acute renal insufficiency due to lower nephron nephrosis; studies of sodium and water balance were continued for longer periods in these patients and in six additional ones. Calculations of extracellular-intracellular partition of water, sodium, and potassium were made in the four patients according to the method of Darrow.

Marked fall in plasma sodium was encountered during the oliguric phase as well as during the diuretic phase. The drop during the oliguric phase was due partly to dilution in extracellular fluid and partly to intracellular migration. The drop during the diuretic phase was traceable to the inability of the damaged kidney to retain sodium. Spontaneous resumption of capacity to conserve sodium tended to occur about five to seven days after the onset of diuresis. The importance of following plasma sodium levels and using these as a guide in the treatment will be stressed. Another cardinal feature in the treatment, namely, the daily calculation of fluid balance and utilization of this as an index to fluid intake, will also be brought out.

The plasma potassium did not rise to toxic levels in any of our cases. In one patient who went through ten days of extreme oliguria following circulatory collapse due to diabetic coma, the plasma potassium remained below toxic levels as a result of intracellular uptake of potassium which was released from endogenous protein catabolism. Impairment in capacity to conserve potassium was demonstrated during the diuretic phase and in some cases necessitated administration of supplementary potassium chloride to combat hypopotassemia.

46. THE EFFECT OF X-IRRADIATION ON ANTIBODY FORMATION

L. O. JACOBSON, M.D., M. E. ROBSON, B.S. (BY INVITATION), E. K. MARKS (BY INVITATION), AND M. C. GOLDMAN, B.S. (BY INVITATION), CHICAGO, ILL.

Hektoen, in 1915, first demonstrated that total body exposure of experimental animals to ionizing radiation suppressed the usual antibody response to antigens injected shortly before or shortly after irradiation. Hektoen's observations led him to ascribe this suppression to the destructive effect of radiation on the lymphatic tissue.

This communication relates an attempt to study the capacity of the rabbit to form antibodies during a period in which all lymphatic tissue of the body, with the exception of the spleen or appendix, is essentially destroyed.

Young adult rabbits were given 800 r. total body x-irradiation (250 kv.) twenty-four hours prior to intravenous immunization with sheep cells (1 c.c. of a 2 per cent suspension). Hemolysin titers were determined on serum obtained before immunization and at seven, fourteen, twenty-one and twenty-eight days afterward. Animals thus irradiated and immunized either developed no demonstrable hemolysin titer or developed titers of 1 to 80 or 1 to 40 which were demonstrated on the twenty-first day and the twenty-eighth day, respectively, after immunization. In the normal animal hemolysin titers of 1 to 5120 were produced by the fourteenth day after the intravenous administration of the antigen, diminishing to titers of circa 1 to 160 by the twenty-eighth day. Rabbits given 800 r. total body x-irradiation exclusive of the surgically mobilized, lead-pro-

tected spleen or appendix produce antibodies to this antigen in a manner comparable to normal nonirradiated control animals except that the maximum titer of 1 to 5120 is usually reached on the twenty-first day rather than on the fourteenth. Surgical mobilization of the spleen or appendix was performed while the animals were under Nembutal anesthesia. In the experiments involving spleen protection, the spleen was drawn through a left upper quadrant incision and placed in a lead box, one-fourth inch thick with an opening for the pedicle only, during the period of irradiation. In the experiments involving appendix protection, the method was similar except that the appendix was drawn through a right lower quadrant incision and a cylindrical lead shield, open at one end, was used.

These experiments corroborate Hektoen's original classic findings that antibody formation is suppressed by total body x-irradation. In addition, it has been demonstrated that if the spleen or appendix of the rabbit is protected by lead shielding during irradiation of the balance of the body, the capacity to produce antibodies to an injected particulate antigen in an essentially normal manner is retained, even though lymphatic tissue elsewhere in the body is largely destroyed.

47. RENAL AND EXTRARENAL DISPOSAL OF CHORIONIC GONADOTROPIN IN THE IMMEDIATE POST-PARTUM PERIOD

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The fate of chorionic gonadotropin in the human being has not been well established. Not more than 20 per cent of chorionic gonadotropin administered parenterally to men appeared in the urine.* Similar experiments in nonpregnant women indicated occasionally much larger urinary recoveries, leading to the view that relatively little destruction or utilization of the hormone occurred in the body.† The foregoing results, however, are not necessarily applicable to the fate of the hormone during pregnancy.

We have studied the renal and extrarenal disposal of chorionic gonadotropin in fifteen pregnant women. Starting immediately on placental delivery, samples of venous blood were taken at three-hour intervals for two or more days. Urine was similarly collected by an indwelling catheter for three or more days. Bioassay of the serum and urine for chorionic gonadotropin was performed by Albert's method.‡ The total circulating hormone was estimated at zero time (immediately after the delivery of the placenta) by multiplying the determined concentration of hormone per milliliter of serum by the estimated serum volume. The total amount of hormone appearing in the urine was obtained by addition of the determined values for all samples.

The mean total circulating hormone for the fifteen patients was 36,245 I. U. The mean urinary excretion was 2,105 I. U. Thus a mean of 5.8 per cent of the active circulating hormone was excreted in the urine; 94.2 per cent of it was disposed of extrarenally, presumably by endogenous inactivation or destruction.

This conclusion was further borne out by analysis of the blood and urine curves following placental delivery and by calculations involving the renal clearance of the hormone. In the immediate post-partum period, the renal clearance was 0.47 ml. per minute. At this rate, the serum would be entirely cleared

*Friedman and Weinstein: Endocrinology, **21**: 489, 1937.

†Bradbury and Brown: J. Clin. Endocrinol. **8**: 1037, 1948; Lloyd and co-workers: J. Clin. Endocrinol. **9**: 268, 1949.

‡J. Clin. Endocrinol. **8**: 619, 1948.

of hormone in 106 hours, or cleared to the extinction point of the bio-assay in 89 hours. The blood disappearance curves have a mean half-life of 3.4 hours, or a time for extinction of 9.3 hours, which is grossly incompatible with the time (89 hours) that would be required for reaching the extinction point if the removal of the hormone from the blood depended entirely on renal excretion. Thus, a major route of disposal of the hormone other than renal excretion of the active hormone is present.

48. THE MEASUREMENT OF THE PERIPHERAL CIRCULATION

A Quantitative Study

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To date, the clinical measurement of the peripheral circulation is made by indirect means only. The instrumental methods most commonly used are skin temperature studies, oscillometry, plethysmography, photelometry, x-ray visualization of the blood vessels, and the use of isotopes.

It is not the purpose of the present paper to evaluate or discuss the relative merits of the various methods but to present a brief report on the results of the measurement of the peripheral circulation by a new method and to discuss the various components of the peripheral records such as amplitude, crest times, diacrotic notch, and the central pulse as related to the peripheral pulse.

Slides will be shown illustrating the quantitative measurement of the pulsatile peripheral circulation in patients with such diseases as hypertensive heart disease, organic occlusive arterial disease, cardiac arrythmias, and organic valvular disease. A few studies on the effects of smoking in sensitive subjects will be presented.

A special adaptor, for the lantern slide projector, has been made for demonstration purposes. By this means it is possible to project on the screen the circulatory changes from any location in the extremities. During the course of the discussion the circulatory changes from a normal subject will be shown, illustrating the speed and ease with which quantitatively calibrated records can be made.

49. COOMBS TITER VARIATIONS IN ACQUIRED HEMOLYTIC ANEMIA

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The erythrocytes from seven of ten patients with acquired hemolytic anemia have shown agglutinability with antihuman serum rabbit serum (direct Coombs test). Of the three instances of hemolytic anemia with negative Coombs tests, one was a severe but transient episode complicating pneumonia and empyema, one had persisted intermittently for six years in an individual with no family history of jaundice and a normal erythrocyte osmotic fragility, and one occurred as the terminal episode in a patient with Hodgkin's disease.

Two patients with Hodgkin's disease, one with carcinomatosis and one with pernicious anemia, experienced hemolytic episodes associated with a positive Coombs test. Agglutination of their erythrocytes occurred only in low dilutions of rabbit serum and decreased or disappeared with the termination of the episode. Because commercially available antiglobulin serum failed to give similar reactions, the specificity of the local serum was checked against erythrocytes from sixty patients obtained as random samples from the blood bank. Only one

sample, erythrocytes from a female patient hospitalized for transfusion for anemia, gave a similar low titer reaction. Erythrocytes from fifteen anemic patients with carcinomatosis, Hodgkin's disease, multiple myeloma, leucemia, Gaucher's disease, hypersplenism, and pernicious anemia, all without evidence of hemolysis, gave negative Coombs tests.

In contrast to the low titer agglutination observed in patients with symptomatic hemolytic anemia, erythrocytes from three patients with acute idiopathic hemolytic jaundice were agglutinated by high dilutions of antiglobulin serum. The direct Coombs titers persisted at high levels in two patients subjected to splenectomy. The patient without demonstrable serum antibody experienced partial remission; the patient with serum auto- and iso-agglutinins was not benefited. A third patient, without splenomegaly, received nitrogen mustard. This did not alter the Coombs titer or the degree of hemolysis and produced a fatal thrombocytopenia.

It is concluded that not all patients with acquired hemolytic anemia have positive Coombs tests, and that potent antiglobulin serum is necessary to obtain positive tests in certain other cases. In those cases with low titer agglutination, the finding of a positive Coombs test was correlated with the presence of a hemolytic episode. In two patients showing high titer agglutination, splenectomy did not lower the agglutination titer of their erythrocytes in antiglobulin serum but did lead to a beneficial decrease in the rate of erythrocyte destruction in one case. Additional study is necessary to determine the effect of detectable serum agglutinins on the rate of hemolysis following splenectomy.

50. CLINICAL EXPERIENCE WITH A NEW ANALGESIC AGENT

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(INTRODUCED BY F. W. MADISON, M.D.)

Clinical trial of a new analgesic, 3-hydroxy-N-methyl-morphinan-hydrobromide (NU-2206), has been carried out. Of thirty-five patients studied, eleven had advanced malignancy, nine were postoperative, and the remaining fifteen had pain from a variety of causes including renal colic, lupus erythematosis, peripheral vascular disease, rheumatoid arthritis, fracture, myocardial infarction, and congestive heart failure. Experience thus far indicates that the drug has approximately three times the analgesic potency of morphine. In seven patients relief of pain was inferior to that obtained when comparable dosage of other analgesics was used. Nausea and vertigo were encountered though less commonly than with morphine, Dilaudid, or Pantopon. When used over a long period of time, tolerance developed and the dosage had to be increased. Euphoria was noted in only four patients. One patient with advanced peripheral vascular disease received fourteen doses of 3 mg. and twenty-six doses of 5 mg. over a period of sixteen days. Following amputation on the sixteenth day, pain ceased and the drug was stopped without withdrawal symptoms. The most striking results were obtained in the group with advanced carcinoma where in many instances pain could be completely abolished without appreciable sedation or respiratory depression even when as large a dose as 20 mg. was given.

No major toxic effects have thus far been observed.

51. THE EFFECTS OF COLD ON MAN: SPECULATIONS ON DISEASES OF COLD TEMPERATE CLIMATES, NUTRITION, AND THE PITUITARY-ADRENAL AXIS

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Investigations on healthy young men exposed to the cold have uncovered adaptative changes which might provide clues for understanding the genesis of some diseases most prevalent in cold temperate climates. For example, it has been established for troops in the field that the voluntary caloric consumptoin is greater the colder the climate. Among men traveling in extreme cold there are, on occasion, alterations in water metabolism and electrolyte balance, creatinuria, retention of ascorbic acid, and other changes reminiscent of those observed in injury and other stress. Recently, metabolic changes were observed in thirty-two heat-acclimatized men abruptly exposed to a cold climate (mean temperature, –26° F.) for twelve days. The results were similar in many respects to those in man after injection of adrenocorticotrophic hormone and to the "general adaptation syndrome" of animals during stress. Statistically significant responses during the first day included hypothermia, diuresis, hemoconcentration, eosinophilopenia and lymphopenia, neutrophil leucocytosis with increase in the percentage of young cells, hyperuricemia, hyperphosphatemia, and hyperkalemia, hyponatremia and hypochloremia, transient retention of sodium and chloride, and diminution of ascorbic acid in the blood. Most of these changes reverted to normal after two days of exposure. However, in the last six days of exposure there were an increase in kidney and adrenocortical activity, a test dose of water being excreted rapidly at very low specific gravity; continued eosinophilopenia and lymphopenia; and continued hyperuricemia and hyperphosphatemia.

Mills and others have shown that diabetes, hyperthyroidism, pernicious anemia, Addison's disease, and leucemia are most prevalent in cold temperate climates, especially so in populations exposed to wide seasonal and diurnal variations in temperature. There are other chronic disorders such as multiple sclerosis, the rheumatic diseases, and certain cardiovascular diseases which have a geographic distribution similar to that of the five diseases just mentioned. In man, acute exposure to cold has been said to precipitate pneumonia, nephritis, and attacks of gout.

We accept the concept that climatic or environmental stresses are related to the genesis of certain disease processes in man. We would alter the hypothesis of Mills and others that the development of many diseases is directly related to the metabolic load imposed by stimulating climates to the hypothesis that exposure to cold stimulates the pituitary-adrenal axis. There may then occur pathologic changes, some of which have already been detected in studies of the "general adaptation syndrome."

52. PATHOLOGIC AND ELECTROCARDIOGRAPHIC STUDY OF THE AURICLES

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(INTRODUCED BY JOHNSON MCGUIRE, M.D.)

By current standards, auricular disease is said to exist when the P wave in any of the standard leads exceeds .12 second in duration, or 3 mm. in height. The validity of these criteria were studied as follows:

1. Two hundred electrocardiograms on normal subjects ranging from birth to 20 years of age were examined. None of the 200 electrocardiograms exceeded the accepted criteria for auricular damage.

2. One hundred electrocardiograms in which the P-wave measurements exceeded the limits mentioned were chosen by random selection. They were classified according to the clinical diagnoses. Abnormal P waves were found in 5 cases of myocardial infarction, 28 cases of hypertensive arteriosclerotic heart disease, 19 cases of rheumatic heart disease, 4 cases of congenital heart disease, 9 cases of cor pulmonale, and in 35 cases comprising a miscellaneous group (beriberi heart disease, thyrotoxic heart disease, etc.). Broad P waves were recorded three times as frequently as tall P waves.

3. Necropsy findings in 122 unselected cases of heart disease were correlated with the P wave in the electrocardiogram. There were 42 cases of myocardial infarction, 28 cases of hypertensive arteriosclerotic heart disease, 25 cases of congenital heart disease, 15 cases of rheumatic heart disease, and 12 cases of cor pulmonale. Pathologic abnormalities of the auricles were found in 58 cases and consisted of dilatation, hypertrophy, intra-auricular thromboses, endocardial thickening, or combinations thereof. P-wave abnormalities were present in 28, or 49 per cent, of these cases. Where electrocardiographic and pathologic findings co-existed, there was no correlation between the type of P-wave abnormality and the particular pathologic change recorded. Electrocardiographic evidence of auricular abnormalities was present in 42 cases of the entire autopsied series. In 14 instances of these 42 cases, or 33 per cent, pathologic confirmation was absent. There were 50 cases in the series of 122 in which neither pathologic nor electrocardiographic evidence of auricular abnormalities was demonstrable.

It was concluded that (1) abnormal P-wave changes may occur in the absence of gross pathologic changes in the auricles; (2) normal P waves may exist in the presence of gross pathologic changes in the auricles; (3) no correlation existed between the auricular chamber involved and the particular lead in which P-wave abnormalities were found.

53. THE PRESENCE OF A PHOSPHATASE IN THE HUMAN AORTIC WALL

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The failure to demonstrate a phosphatase in the human arterial wall has been a subject of significance in the discussion of the pathogenesis of arterial calcification. Since previous investigations appear to have been performed only at alkaline reactions, a study was undertaken covering a wider pH range.

After removal of the adventitia and the external part of the media from human aortas obtained at autopsy, the remaining part of the tissue was ground

finely with water in a Pyrex grinder and the samples were centrifuged. For study of the enzymatic activity portions of the supernatant solution were added to citric acid sodium hydroxide and Veronal sodium carbonate buffers, covering the pH range 3.1 to 10.1. Disodium phenyl phosphate was used as a substrate. Duplicate samples, controls, and blanks were run at each pH level examined. The reaction was stopped by the addition of trichloroacetic acid and the phenol content of the filtrates determined by Folin and Ciocalteus's method. The enzymatic activity was estimated by subtracting the color of the controls from that of the samples.

The studies showed a significant phosphatase activity with a maximum at pH 5.7 to 5.8. At this optimal pH level a fair proportionality was observed between the amount of supernatant fluid used and the phosphatase activity and between the reaction time and the enzyme activity.

54. THE DIAGNOSTIC VALUE OF HIGH PRECORDIAL LEADS

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High precordial leads taken at the intersections of vertical lines through the V_3, V_4, V_5, and V_6 positions with a horizontal line at the junction of the third interspace and sternum have been obtained on approximately 4,000 patients. The findings in these leads were correlated with those in the customary precordial and Goldberger limb leads and with the pathologic findings in 300 cases that came to autopsy.

Cardiac rotation appeared to have a greater influence on the QRS pattern in high precordial leads than in those taken in the usual positions. Clockwise rotation displaced the transitional zone farther to the left in high than in the customary precordial leads and often led to the registration of an RS pattern, typical of the potential variations of the epicardial surface of the right ventricle, in leads high in the axilla as well as in a V_L. Counterclockwise rotation tended to shift the transitional zone farther to the right in the high than in the routine precordial leads.

High precordial leads were of particular value in the detection of infarcts localized to the basal portion of the anterior or lateral walls of the left ventricle, as demonstrated by pathologically proved cases with diagnostic signs in high precordial but not in the routine precordial or limb leads.

High precordial leads were also of value in the estimation of the basilar extent of anterior or lateral apical infarcts, as illustrated by cases of localized anterolateral apical infarction, showing diagnostic patterns in the customary but not in the high precordial leads and extensive anterolateral infarction with diagnostic patterns at both levels. The differentiation from high precordial lead patterns due to right and left ventricular hypertrophy and right and left bundle branch block is illustrated.

55. STUDIES ON THE RENAL TUBULAR TRANSPORT MECHANISM FOR GLUCOSE

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GORDON JOHNSON, B.S., AND ELAINE GIANAS, R.Ph., CHICAGO, ILL.

(INTRODUCED BY ROBERT W. KEETON, M.D.)

Recent observations by Conn and co-workers indicate that the administration of adrenocorticotrophic hormone (ACTH) to normal human subjects results in a diabetic state. In addition to hyperglycemia, ACTH is be-

lieved to decrease renal tubular resorption of glucose. Glutathione antagonizes this effect of ACTH presumably by reversing the inhibitory action of ACTH on tubular transport mechanisms.

Hyperglycemia has been reported to effect adrenal-cortical discharge as reflected by a fall in the lymphocyte count in the rat and also in man. Thus hyperglycemia in itself can be considered as a stress phenomenon.

To obtain quantitative data on the effect of adrenal-cortical discharge on the tubular mechanism for glucose, it was planned to do Tm_g determinations in trained dogs before and after the administration of epinephrine. A preliminary experiment resulted in a 70 per cent drop in the Tm_g and a 77 per cent drop in the eosinophil count, following the intravenous infusion of epinephrine (5 gamma per kilogram per minute for one hour). Eight periods, fifteen to twenty minutes in duration, were conducted in each experiment over a five-hour period of glucose infusion. The Tm_g values for the last two to three periods were within normal limits as reported for the dog (200 to 300 mg. per minute). In five of six dogs, the initial two to three periods showed depressions of the Tm_g ranging from 33 to 59 per cent. Tm_g values as low as 92 mg. per minute have been obtained under these conditions despite glucose loads of 1.5 to 2.0 times the animal's normal Tm_g level. In contrast to the gradual rise in Tm_g as a given experiment progressed, the eosinophil count fell continuously to a low value at the completion of each run.

The marked depressions of tubular resorption of glucose seemed to coincide with high environmental temperatures. This is of interest since it is known that heat stress will fire the pituitary-adrenal mechanism. It is postulated that environmental heat stress in combination with the stress of hyperglycemia may have been responsible for the anomalous Tm_g observations noted in this series of dogs.

56. AN INDIRECT, QUANTITATIVE METHOD FOR THE ESTIMATION OF HEPARIN ACTIVITY IN VITRO: CLINICAL APPLICATION

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Evidence has been presented which suggests that some hemorrhagic states are due to the presence of excessive amounts of heparin in the blood. In such conditions the antiheparin agents, protamine sulfate and toluidine blue, may be of great value clinically. A laboratory procedure which will permit the selection of patients for this type of therapy and which can be used to control such therapy is desirable. A method is described which utilizes a modified "one-stage prothrombin time" type test. With this technique it is possible to detect the presence of heparin in amounts as small as 3.5 μg per milliliter of plasma. Under the conditions of this test the ratio of the concentration of protamine sulfate required to neutralize heparin added in vitro or in vivo is 1.5:1. This ratio appears to be a constant. When the amount of protamine sulfate required to neutralize a certain amount of added heparin is greater than that predicted by this ratio, it is concluded that the excess protamine neutralizes endogenous heparin. The utility of the test in the protamine sulfate therapy of certain hemorrhagic states is illustrated by case histories. In practice it has been possible to predict which patients will be benefited by the use of antiheparin agents. It has also been possible to determine the adequacy of the treatment, even in the absence of frank bleeding.

57. THE EFFECT OF ATROPINE SULFATE AND DIBUTOLINE ON THE NOCTURNAL GASTRIC SECRETION IN MAN

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The effect of atropine and of atropine and adrenaline in oil on the fasting gastric secretion was studied in forty-five patients hospitalized for peptic ulcer. Atropine administered parenterally in dosages of 1.0 to 2.0 mg. every 3 to 4 hours exerted a variable effect. A depression in acid secretion occurred in approximately 33 per cent of the studies; the effect was of short duration. Increases in gastric secretion were observed in approximately 26 per cent of the studies. Similar results were obtained with 2.0 mg. doses. The action of atropine upon the parietal cell appeared to be related to the state of activity of the cells at the time of administration of the drug. Of the patients with duodenal ulcer in whom a reduction was obtained, the secretory rate in 66 per cent was less than the average hourly rate observed in such patients. However, a depression in gastric secretion was not obtained uniformly in all individuals with low rates of secretion. The reduction in output of acid in individuals with high secretory rates was associated with severe symptoms of atropine toxicity. Atropine also was administered in doses of 1.0 mg. simultaneously with 2.0 mg. of adrenaline in oil. No additive effect was noted insofar as the volume of gastric secretion was concerned. However, the concentration and output of acid were reduced more frequently by the combination of drugs than with atropine alone.

The effect of a pharmacologically related compound, Dibutoline (Dibutylurethane of dimethyl-ethyl-β-hydroxyethyl ammonium sulfate—Merck) on the nocturnal gastric secretion was studied in fifteen individuals. Dosages of 10 mg. given intramuscularly every four hours during the night yielded results similar to those obtained with atropine. Although a decrease in the output of acid was observed more frequently with Dibutoline, the depressant effect, whenever it occurred, likewise was variable and of short duration.

58. STUDIES OF BLOOD COAGULABILITY AS INFLUENCED BY DIGITOXIN

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Digitoxin was administered to all subjects used in this study by either the oral or parenteral route. In all instances studies of the coagulation mechanism were performed both before and after digitalization with digitoxin. All subjects used presented no evidence of cardiac decompensation or of thromboembolic disease.

The bleeding time, prothrombin time, and coagulation time (Lee and White) were determined in five patients. Two of these patients received 0.6 mg. digitoxin intravenously and three received 1.2 mg. digitoxin intravenously. The aforementioned studies were then determined at intervals of five to ten minutes, one hour, four hours, seven hours, and twenty-four hours. In no instance was there any significant decrease in the bleeding time, coagulation time, or prothrombin time.

Eleven patients were then studied with heparin tolerance estimations before and after receiving a full digitalizing dose (1.6 mg.) of digitoxin administered orally over a twenty-four hour period. No significant change in the

heparin tolerance curves was apparent after digitalization when the test was performed according to the method described by de Takats and associates. In order to make the method more sensitive, it was modified by using the Lee and White coagulation time. With this modification, six determinations on five patients revealed an increase in heparin tolerance, and seven determinations on six patients revealed a decrease in heparin tolerance. It is suggested that the wide variations in these data are due to variations intrinsic in the method rather than to the effect of digitoxin.

The problem was then approached by studying the prothrombin times by a modified Quick one-stage technique in twelve subjects. Following digitalization with digitoxin, there were no significant alterations in the prothrombin times in any instance. After digitalization, the subjects were Dicumarolized in the usual fashion. No inhibition of the effect of Dicumarol by digitoxin as measured by the effect on the prothrombin activity was evident.

Three patients were given Dicumarol so that prothrombin activity was reduced to a range of 10 to 30 per cent. After stabilization of prothrombin activity by the administration of maintenance doses of Dicumarol, each of the three patients was digitalized with 1.6 mg. of digitoxin given orally. In none of the three cases did the digitoxin appear to interfere with the effect of Dicumarol as reflected by the prothrombin activity.

Finally, the problem was approached by the determination of the coagulation time both by the conventional Lee and White technique and in tubes treated with silicone for the purpose of simulating the endothelial lining of the vascular network. Ten patients were studied by this method for a three-day control period. It was noted that there was considerable variation in the coagulation times in the same individual from day to day. This variation was noted in both techniques but was especially evident in the technique employing test tubes treated with silicone. These patients were then digitalized by the oral adminisraion of 1.6 mg. of digitoxin, and they were placed on daily maintenance doses of this drug. The coagulation time was studied daily for three days according to the previously mentioned techniques. Again there were no consistent changes in the coagulation time as measured by either technique following the administration of digitoxin.

It is concluded, therefore, that the methods used in this study have demonstrated that digitoxin, administered either orally or intravenously, has no effect on the coagulation mechanism of the blood. Therefore, if digitalization is indicated, this study suggests that there should be no hesitation in carrying out this therapeutic procedure with a view that thromboembolism may result therefrom.

59. THE USE OF VIODENUM IN THE TREATMENT OF ULCERATIVE COLITIS

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(Introduced by Armand J. Quick, M.D.)

A continuous search is being made to find an adequate means of combating the etiological factors of chronic idiopathic ulcerative colitis. Among the more recent additions to our medical armamentarium is Viodenum. This substance is whole desiccated and defatted duodenum, which is believed to contain certain antisecretory and antiproteolytic factors. Thirteen patients with chronic ulcerative colitis in acute exacerbation were treated with Viodenum in addition to the commonly used therapeutic measures. Tablets were administered

only after powder had been given in the initial phase of the treatment and until the acute flareup had subsided. The results are as follows: (1) one patient showed no improvement; (2) exacerbation of the disease was noted in three instances, in one of which the tablets passed through the bowel without disintegration; (3) two patients were forced to discontinue treatment because of intolerance as manifested by nausea, diarrhea, and cramps; (4) definite clinical improvement was observed in six patients; (5) no definite opinion could be reached in two.

60. THE EFFECT OF TETRAETHYLAMMONIUM BROMIDE ON THE CARDIAC OUTPUT OF NORMOTENSIVE AND HYPERTENSIVE PATIENTS

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In a previous study we have shown that the fall in arterial blood pressure of normal and hypertensive patients induced by spinal anesthesia is not associated usually with a significant fall in cardiac index. This evidence has been used to support our belief that the cause, or at least the sustaining cause, in essential hypertension is an increased vasomotor tone. In attempting further to elucidate the pathogenesis of essential hypertension, tetraethylammonium was used to lower the blood pressure in patients with essential hypertension, and cardiac output studies were made by the direct Fick method.

Sixty-one cardiac output measurements were made on nine normotensive and eleven hypertensive patients before, during, and after the intravenous injection of tetraethylammonium bromide. The twenty patients were divided into four groups: sedated and unsedated hypertensives, sedated and unsedated normotensives.

In both the sedated and unsedated normotensives tetraethylammonium produced a fall in blood pressure but no significant fall in cardiac index. After recovery from the tetraethylammonium, as shown by the disappearance of tachycardia and the return of the blood pressure to or toward control levels, the cardiac index remained lower than either the control level or the level obtained during the tetraethylammonium effect.

In the unsedated hypertensive group the control cardiac indexes were slightly lower than in the normotensive or sedated hypertensive groups. During the tetraethylammonium effect the cardiac index showed little change and in some cases rose to a level above that of the control value.

In the sedated hypertensive group there was little fall in the cardiac index during the tetraethylammonium period in spite of marked falls in the blood pressure. In no case in the whole series did the cardiac index fall below the normal limit during the tetraethylammonium effect.

In both normotensive and hypertensive patients a sympathicolytic agent such as tetraethylammonium may produce a fall in blood pressure without significant fall in cardiac index.

(To be concluded in the December issue.)